

Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 37.

JANUARY, 1938.

No. 4.

SECTION MEETINGS

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Northwestern University December 23, 1937

MINNESOTA

University of Minnesota December 15, 1937

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New York Academy of Medicine January 19, 1938

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Further Evidence for a Mammogenic Hormone in the
Anterior Pituitary.*†

E. T. GOMEZ AND C. W. TURNER.

*From the Department of Dairy Husbandry, Missouri Agricultural Experiment
Station.*

The effectiveness of the ovarian hormones, estrogen and progestin, in stimulating the growth of the mammary glands has been demonstrated in many species. Until recently it was assumed that

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 546.

† Aided in part by a grant from the International Cancer Research Foundation.

their action was direct. When these hormones were found to be ineffective in hypophysectomized animals,¹ it appeared that the pituitary must play a more important rôle in the growth of the gland than had previously been suspected. Gomez, Turner, and Reece² presented the first evidence for the presence in the pituitary of a mammary gland growth-promoting factor not associated with the lactogenic hormone. This new pituitary principle will be called the "mammogenic hormone". There are probably 2 mammogenic factors,³ the duct growth factor being stimulated by estrogen and the lobule-alveolar growth factor by estrogen and progesterone. As the growth of the udder is very rapid, especially during the first half of pregnancy, the effectiveness of the pituitaries of such animals in stimulating the growth of the mammary gland was determined.

Studies with Rabbits. Four sexually immature female rabbits were castrated 4 weeks prior to the initiation of the experiment. A control gland removed from each of these animals showed only a rudimentary duct system. The first rabbit was injected for 25 days with 50 mg. daily of fresh anterior pituitary from pregnant cattle. At autopsy the mammary glands showed growth and development comparable with that observed at a late stage of pregnancy.

The second rabbit received 20 mg. daily for 30 days of acetone dried anterior pituitary powder[‡] from heifers pregnant not more than 140 days (pregnancy in cattle being 280 days). The mammary glands at that time were comparable to those at the end of pseudopregnancy, being fully grown but not showing signs of lactation. The third rabbit received daily for 30 days 20 mg. of acetone-dried powder from heifers pregnant over 140 days. The glands showed only an extensive growth of the duct system. Assay of these preparations for lactogenic and thyrotropic hormones has been reported.⁴ It should be pointed out that while the pituitary powder from the non-pregnant heifers contained considerable lactogenic hormone, it failed to stimulate the growth of the mammary gland.

The nipples of all experimental rabbits remained infantile, which confirms our previous observations on the hypophysectomized guinea

¹ Gomez, E. T., and Turner, C. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **34**, 320; **36**, 287.

² Gomez, E. T., Turner, C. W., and Reece, R. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 286.

³ Gomez, E. T., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.*, 259, 1937.

[‡] We are indebted to Dr. R. P. Reece for the acetone dried cattle pituitary powders used in this study.

⁴ Reece, R. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.* 266, 1937.

fig.² It appears that estrogen stimulates the growth of the nipple directly while the mammogenic hormone stimulates the growth of the gland.

Studies with Rats. Eight sexually immature female rats were castrated not less than 4 weeks preceding the beginning of the experiment. Control mammary glands removed immediately preceding the initial injection consisted of short slender ducts which had a few almost naked twig-like branches projecting from them. Six of these rats were given daily injections of 10 mg. of fresh anterior pituitary from pregnant cattle and the remaining 2 with 10 mg. of fresh pituitary from non-pregnant heifers. Three of the rats which received pregnant cattle pituitaries were sacrificed after 10 days. Examination of the mammary glands of these animals showed extensive arborization of the duct system with characteristic enlargement or budding of the peripheral ends. Lobules were observed in the region of the older ducts in the proximity of the nipples. The remaining animals were sacrificed after 25 days. The mammary glands of these animals showed an extensive arborization of ducts and proliferation of the lobule-alveolar system comparable to that observed in rats pregnant about 18 days. Milk secretion was evident in the lumina of the large ducts. The 2 rats which received pituitaries from non-pregnant heifers showed no additional growth of the duct system of the mammary gland.

Summary. Further evidence is presented indicating that the pituitary gland is the seat of production of a factor or factors which stimulate the growth of the duct and lobule-alveolar systems of the mammary gland. This principle, called the "mammogenic hormone," is present in the pituitaries of cattle when pregnant. The growth of the mammary glands of castrated female rabbits and rats was stimulated by the daily injection of such pituitaries. That this principle is not identical with the lactogenic hormone is indicated by the lack of response with non-pregnant cattle pituitaries containing considerable amounts of the lactogenic hormone.

Urinary Output of Nitrogen as Influenced by Intravenous Injection of a Mixture of Amino-Acids.

ROBERT ELMAN. (Introduced by Evarts A. Graham.)
(With the technical assistance of Delores Jean Fischer.)

*From the Department of Surgery, Washington University School of Medicine, and Barnes Hospital, St. Louis, Mo.**

Evidence that a mixture of amino-acids injected intravenously produced rapid regeneration of serum protein was recorded previously.¹ The present communication deals with observations on the urinary excretion of nitrogen as influenced by the administration of amino-acids in this way. Previous experiments by Henriques and Andersen² have indicated that a positive nitrogen balance may be attained even when all of the nitrogen intake was injected intravenously in the form of an enzymatic digest of meat. These authors used one goat, but the preparation was not completely hydrolyzed to amino-acids inasmuch as it contained 15% of its nitrogen in the form of peptides.

In the present experiments the mixture of amino-acids was obtained by the acid hydrolysis of casein, as previously described.¹ Such a preparation contains no tryptophane, which is destroyed during the hydrolysis and is low in cystine. This incomplete basic mixture, however, served as a control for the observations in which 2% of each of these 2 essential amino-acids were added to form the "complete" mixture.†

Healthy, adult, short-haired dogs were used. They were kept in metabolism cages, allowed water *ad libidum*, starved for a few days and then given, twice daily, by gavage a solution of 12½% sucrose in normal saline. The amount of sucrose administered in this way was calculated to yield about 50 to 60 calories per kilo of body weight. The same amount was given daily in each case. This procedure tended to spare protein breakdown and thus produced a much lower nitrogen output in the urine than occurs during starvation. Moreover, it acted as such a good diuretic that large, relatively

* Aided by a grant from the Louis D. Beaumont Fund.

¹ Elman, R., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 867.

² Henriques, V., and Andersen, A. C., *Z. f. physiol. chemie*, 1913, **88**, 357.

† The hydrolyzed casein was kindly furnished by Mead, Johnson and Company. The added tryptophane and cystine were obtained from Eastman Kodak Company. Each of the added amino-acids was levorotatory.

uniform amounts of urine were passed each 24 hours; catheterization was thus deemed unnecessary. The feces under the conditions of the experiment were scanty and infrequent; their nitrogen content was assumed to be constant and slight and they were therefore not saved for analysis. The 24-hour urine was collected in receptacles containing toluol to prevent fermentation and sufficient acid to neutralize any ammonia. All experiments were carried out in consecutive 3-day periods. The total nitrogen of the urine was determined by direct digestion and Nesslerization. Following the preliminary starvation period, the dogs were observed for from 3 to 7 days during which period the output of urinary nitrogen dropped considerably; experiments were begun only after the urinary nitrogen approached a relatively uniform level. The amino-acids were given in a 10% solution containing also 10% glucose. Ten to 20 cc. were injected intravenously each hour until the total daily dose was given; this usually required 6 to 10 hours and produced, in no case, any evidence of toxicity. Previous observations had shown that with this rate of injection very little of the amino-acids appeared in the urine (*i. e.*, under 10%).

The findings, as shown in Table I, represent 15 days' observation on 2 dogs; they were chosen from experiments on 7 dogs, all of which showed similar results. It will be noted that N-balance was immediately achieved during the periods in which the complete mixture of amino-acids was injected; this was possible even though the amount of nitrogen administered was slightly in excess of the amount excreted in the preceding period. Thus, in spite of the large amount of nitrogen injected there resulted only a slight increase in the urinary nitrogen excreted, indicating a very high degree of retention of the injected amino-acids. In dog B70 3 gm. of nitrogen were excreted each day during the first 3 days; 3.11 gm. were excreted daily the next 3 days during which 3.16 gm. of nitrogen as amino-acids were injected. In dog B9 the results were similar, *i. e.*, while 1.58 gm. of nitrogen were being given daily the output was the same, whereas in the 3 preceding days when no amino-acids were given the output was only slightly lower, 1.47 gm. The immediate achievement of nitrogen balance has not been the case in many experiments by others in which protein or amino-acids were fed by mouth. The authors mentioned above,² however, did achieve such an immediate effect; they are apparently the only other observers who studied nitrogen balance during the intravenous administration of amino-acids.

In contrast to these findings with the complete mixture of amino-

TABLE I.

Day	Urinary Output		Daily Aver. (gm. N)	N-Intake Daily Injections Amino acids (gm. N)	N-Balance	Remarks
	Amt. (cc.)	Total N (gm.)				
1	900	2.79				Dog B70
2	600	2.52				Wt. 10.1 kg.
3	820	3.70	3.00	0.0	-3.00	125 gm. sucrose daily
4	850	3.21				Amino-acid mixture
5	1200	3.71				containing 2% tryptophane and added
6	1100	2.40	3.11	3.16	+ .05	cystine
7	1000	2.50				
8	850	2.47				
9	810	2.51	2.50	0.0	-2.50	
10	750	3.45				Incomplete (basic)
11	850	4.25				amino-acid mixture
12	900	3.33	3.68	3.16	— .52	
13	830	3.32				
14	750	1.80				
15	670	2.14	2.42	0.0	-2.42	Wt. 8.6 kg.
1	600	1.44				Dog B9
2	600	1.14				Wt. 6.6 kg.
3	800	1.52	1.37	0.0	-1.37	100 gm. sucrose daily
4	1100	2.31				Incomplete (basic)
5	1000	1.90				amino-acid mixture
6	1000	2.00	2.07	1.29	— .78	
7	950	1.90				
8	490	1.12				
9	700	1.40	1.47	0.0	-1.47	
10	1000	1.60				Amino-acid mixture
11	1200	1.56				containing 2% tryptophane and added
12	1000	1.60	1.58	1.58	0.0	cystine
13	700	1.40				
14	900	1.89				
15	750	1.72	1.67	0.0	-1.67	Wt. 6.0 kg.

acids are those with the basic mixture in which no tryptophane or cystine was added. In neither dog was N-balance achieved though the amount of nitrogen injected was about the same. Indeed, of the injected amino-acids only about one-half was retained in one case and but four-fifths in the other. It is of interest to note too that the increased nitrogen output which occurred during the days of injection persisted for the next 24 hours into the period during which no injections were given. This did not occur when the complete mixture was injected.

It should be emphasized that these observations are not concerned with the biological value of hydrolyzed casein inasmuch as a condition of minimum endogenous nitrogen metabolism was probably not achieved in these dogs. Moreover, the period of observation was too short for such a study. The experiments are concerned only with the question as to whether amino acids injected intravenously this way are efficiently utilized by the body, particularly in the synthesis of tissue protein. It seems clear that if the amino-acids were retained as such that this would take place at approximately the same degree regardless of whether or not the mixture contained the 2% added tryptophane and cystine. That a high degree of retention occurred only with the complete mixture is evidence that synthesis of protein had probably taken place.

Summary. Adult dogs, fed only with sucrose, were immediately brought into N-equilibrium upon intravenous injection of a complete mixture of amino-acids combined with glucose. This appears to demonstrate a high degree of nitrogen retention particularly since nitrogen balance was attained with the administration of an amount of nitrogen but slightly in excess of the previous level of excretion. This was impossible if the mixture did not contain added tryptophane and cystine; under such conditions a large part of the injected nitrogen appeared in the urine. These observations would seem to indicate that the mixture of amino-acids used, as prepared by the acid hydrolysis of casein plus 2% tryptophane and added cystine, is efficiently utilized by the body, probably in the active synthesis of body protein.

9661 P

The Blood Diastase in Acutely Perforating Peptic Ulcers.*

J. G. PROBSTEIN, S. H. GRAY AND P. A. WHEELER.

From the Laboratory of the Jewish Hospital and the Snodgrass Laboratory, City Hospital, St. Louis, Mo.

Meyer and Antman¹ recently reviewed the meagre literature on the blood and urinary diastase in peptic ulcers. The results were inconclusive. They reported 3 cases of high urinary diastase in patients with peptic ulcers involving the pancreas. One case was

* This work aided by the David May Fund.

¹ Meyer, K. A., and Antman, L., *Am. J. Surg.*, 1936, **33**, 307.

proved by autopsy; the other 2 cases were treated medically, the diagnosis being based upon the clinical picture and X-ray examination.

The fact that high diastase is as a rule associated with acute pancreatic disease has led to the idea that the situation of perforating peptic ulcers, especially in relation to the pancreas, might be indicated by the level of the blood diastase. This report is based on a study of 9 cases of acute perforating ulcers, all of whom came to operation or postmortem examination. The situation of the lesions was verified. The diastase was determined by Somogyi's method.²

TABLE I.
Blood Diastase

Case	Pre-operative	Post-operative	Involvement of Pancreas
1	214	130	Yes
2	200	150	"
3	600-402-150-90	No operation	"
4	198		"
5	84		No
6	107	73	"
7	67	40-40-76	"
8	31		"
9	59		"

With 80 to 180 as the normal range of diastatic values,² it can be seen that in the 4 cases in which the ulcer involved the pancreas the blood diastase was elevated. In 3 of the 4 cases the increase in diastase was moderate; while the fourth was high, coming within the range of the figures usually encountered in acute pancreatitis. Of the 5 cases in which the ulcer was not near the pancreas, 2 showed figures within the normal range, while 3 were definitely subnormal. According to Somogyi, low diastase is frequently associated with impaired liver function.² This may indicate some relationship between ulcer and biliary tract-liver disease.

We may tentatively say that a normal or low blood diastase rules out not only acute pancreatitis but a peptic ulcer perforating near the pancreas. A high diastase in a case of sudden sharp pain in the upper abdomen usually indicates pancreatic involvement. A moderate increase during the height of an attack, would tend to indicate a perforating peptic ulcer. A very high diastase, while usually associated with acute pancreatitis, may mean an ulcer acutely perforating in or near the pancreas, as one of our cases proved. This possibility must be taken into consideration in the diagnosis of acute pancreatitis, since the treatment is different.

² Somogyi, M., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **32**, 538.

Diastase determinations may therefore be of distinct help in the differential diagnosis of acute conditions of the upper abdomen, but it must be remembered that they only supplement clinical judgment.

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Effect of Insulin on Carbohydrate Tolerance of Nondiabetic Individuals.

J. T. MAHER AND MICHAEL SOMOGYI.

From the Laboratories of the Robert Koch Hospital and the Jewish Hospital of St. Louis.

One of us (Somogyi)¹ has shown that in diabetic patients the continued overdosage of insulin, *i. e.*, the administration of quantities sufficient to cause hypoglycemias, gradually diminishes the carbohydrate tolerance of the patient and aggravates the diabetic condition. In this paper we call attention to the fact that insulin when administered to nondiabetic individuals, exerts a similar effect.

This observation was made on nondiabetic tuberculous patients, who were treated with insulin with the purpose of inducing an increase in their body weight. The procedure in this work differed from others described in the literature in 2 respects. In the first place, the amount of insulin used was very small. In 2 cases only 10 units were given daily, injected in 5-unit doses before the noon and evening meals, while the remaining patients received at the beginning 10, and subsequently 20 units daily, always administered in 2 equal doses. In the second place, the duration of the treatment in this experiment was much longer than in others reported; the observations extended in 5 cases over a period of 6 months, and in one case over 12 months.

In each case glucose tolerance test was performed a short time before the beginning of the treatment and a second test after insulin injections had been continued for approximately 6 months; in one case a third test was carried out after 12 months of uninterrupted treatment. In the tolerance tests uniformly 100 gm. of glucose was given orally and the blood sugar was determined at hourly intervals by the Shaffer-Hartmann method, using copper reagent No. 50 of Shaffer and Somogyi.² The blood samples were depro-

¹ Somogyi, M., *PROC. SOC. EXP. BIOL. AND MED.*, in press.

² Shaffer, P., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 695.

teinized with Somogyi's zinc method³ which removes nonfermentable reducing substances so that the analysis furnishes true sugar values (which are about 20 mg. % lower than the apparent sugar values obtained with the Folin-Wu method). The results are presented in Table I. It may be seen from these figures that following the insulin treatment the carbohydrate tolerance of all of the subjects has undergone marked deterioration, so that their response to glucose tolerance tests became identical with that of diabetic patients. It is noteworthy that the change in 2 of the 6 patients, Kate R. and Jessie H., who received only 10 units of insulin per day, was of the same order of magnitude as in the others.

TABLE I.
Deterioration of the Carbohydrate Tolerance of Nondiabetic Subjects as the Result of Insulin Injections.

Name and age of patient	Date of test	Time, hr.					Daily dosage of insulin
		0	0.5	1	2	3	
		Blood Sugar, mg. % (true sugar)					
Ella, 42	8/31/36	76	178	137	105	81	10 U., 10/22/36 to 2/20/37, 20 U., from then on
	4/26/37	80	191	191	116	60	
Jessie, 33	9/11/36	101	153	97	71	54	10 U., 9/12/36 to date of tolerance test
	5/3/37	100	167	218	178	95	
Mary, 32	9/1/36	78	125	177	135	87	10 U., 10/22/36 to 12/10/36, 20 U., from then on
	5/3/37	85	204	253	182	101	
Kate K., 38	9/1/36	74	142	97	67	62	10 U., 10/22/36 to 12/14/36, 20 U. from then on
	4/28/37	78	196	189	141	45	
Kate R., 32	8/31/36	79	225	178	161	50	10 U., 10/22/36 to date of second tolerance test
	4/26/37	94	244	233	222	85	
Dorothy, 35	9/1/36	74	145	124	63	47	10 U. 10/22/36 to 1/22/37, 20 U. from then on
	4/28/37	74	176	318	218	107	
	10/7/37	93	—	329	308	154	

The progressive character of the deterioration is well illustrated in the behavior of Dorothy M. Before subjected to insulin injections her response to the glucose tolerance test was definitely non-diabetic. After 6 months of treatment, during the first half of this period with 10, the second half with 20 units of insulin per day, her response became distinctly that of a diabetic, and after another 6 months of injections, with 20 units of insulin daily, her carbohydrate tolerance exhibited further deterioration.

Wilder, Smith and Sandiford⁴ were the first investigators to ob-

³ Somogyi, M., *J. Biol. Chem.*, 1930, **86**, 655.

⁴ Wilder, R. M., Smith, F. H., and Sandiford, I., *Ann. Int. Med.*, 1932, **6**, 724.

serve this phenomenon when studying the influence of insulin upon the body weight of nondiabetic, obese individuals. Two patients of these authors received 55 units daily, one for 12, the other for 25 days. At the end of the treatment it was found in both cases "that responses to glucose tolerance tests were obtained typical of diabetes mellitus."

Clark, Gibson and Paul⁵ employed insulin injections on a number of nondiabetic patients with the purpose of increasing their sub-normal body weight. They injected gradually increasing doses, starting with 15 units daily and raising it frequently to 75 units. In 3 cases glucose tolerance tests were performed immediately before the beginning of the treatment and the day after discontinuation of the injections. One of the patients received 15 to 45 units daily over a period of 20 days, the second 15 to 54 units for 20 days, and the third 15 to 75 units over a period of 45 days. All 3 cases showed deterioration of their carbohydrate tolerance, and in one case the progressive aggravation of the process also was illustrated.

Both groups of workers point out the temporary character of this condition caused by insulin. After discontinuation of the injections the tolerance tests show a gradual return in the direction of normal, but after 8 days of recovery (the changes were not followed longer) the glucose tolerance still was distinctly inferior to that before treatment. We intend to follow the changes in our patients over longer periods of time after stoppage of the treatment; it is of interest to observe the course of recovery after the prolonged action of insulin to which our patients were subjected.

On the basis of these findings it is only to be expected that schizophrenic patients, treated often with very large quantities of insulin, should respond with diminished carbohydrate tolerance. Studies in progress show this to be the case. (This report was presented before the Missouri Section of the Society for Experimental Biology and Medicine last October. Since then Looney and Cameron⁶ published observations made on schizophrenic patients which agree with ours.)

This phenomenon, in our opinion, is closely related to that observed on diabetic patients who are treated with excessive doses of insulin.¹ In the case of the diabetic any amount of insulin that

⁵ Clark, B. B., Gibson, R. B., and Paul, W. D., *J. Lab. Clin. Med.*, 1935, **20**, 1008.

⁶ Looney, J. M., and Cameron, D. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **37**, 253.

depresses the blood sugar below the fasting level represents an overdose which entails high degrees of hyperglycemia, *i. e.*, a further diminution of carbohydrate tolerance. In the case of the non-diabetic subject, whose pancreas supplies adequate amounts of insulin, any added quantity of extraneous insulin represents an excess and leads to deterioration of the carbohydrate tolerance just as overdosage does in the diabetic.

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Blood and Spinal Fluid Magnesium and Calcium Levels in Epilepsy and Convulsive States.*

DAVID M. GREENBERG AND ROBERT B. AIRD. (With the assistance of Muriel Boelter.)

From the Division of Biochemistry and the Department of Surgery, University of California Medical School.

Certain of the constituents of blood and spinal fluid in cases of epilepsy have been subjected to analysis by a number of investigators in order to test the hypothesis that the incidence of convulsions in this condition is associated with a disturbance of the ion balance of the body. No significant variation from normal has been found in the calcium content of the blood and spinal fluid in epileptics.¹⁻⁴

Some time ago Hirschfelder and Haury⁵ reported that they observed low magnesium and high potassium levels in the blood plasma in a high percentage of epileptic subjects during convulsive attacks.

To our knowledge virtually no information is available on the magnesium content of the spinal fluid and red blood corpuscles in epilepsy.

Since the blood and spinal fluid of epileptic subjects were available to the authors, a series of analyses was undertaken on the composition of the blood and spinal fluid of subjects afflicted with epilepsy

* Aided by a grant from the Christine Breon Fund for Medical Research.

¹ Hamilton, B., *J. Biol. Chem.*, 1925, **65**, 101.

² Osnato, M., Killian, J. H., Garcia, T., and Mattice, M. R., *Brain*, 1927, **50**, 581.

³ Katzenelbogen, S., *J. Nerv. and Ment. Dis.*, 1931, **74**, 636.

⁴ Scott, M., and Pigott, A. W., *Arch. Neurol. and Psych.*, 1936, **36**, 596.

⁵ Hirschfelder, A. D., and Haury, V. G., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 40.

and convulsive conditions. The cerebrospinal fluid was collected in the course of encephalography.

Determinations were made of the levels of plasma, red corpuscle and spinal fluid magnesium, serum and spinal fluid calcium, and spinal fluid inorganic phosphorus. Magnesium was determined by the method of Greenberg and Mackey,⁶ calcium by a modification of Kirk and Schmidt's procedure,⁷ and inorganic phosphorus according to Fiske and Subbarow's method.⁸

TABLE I.

Calcium, Magnesium, and Inorganic Phosphorus Levels in the Blood and Cerebrospinal Fluid in Epilepsy and Convulsive States.

Element	No. of Subjects	Extreme Variation	Arithmetical Mean	Stand. Dev.	Coeff. of Variation
		mg. per 100 ml.	mg. per 100 ml.	mg. per 100 ml.	%
Plasma Mg	22	1.55-4.0	2.67	0.45	17
Red Corpuscle Mg	27	5.15-15.5	7.72	2.9	38
Serum Ca	20	8.15-13.4	10.35	1.3	12.5
Spinal Fluid Ca	24	4.75-6.25	5.30	0.4	7.5
Spinal Fluid Mg	17	1.0-3.95	2.93	1.15	39
Spinal Fluid Inorganic P	19	1.35-2.10	1.57	0.7	45

A statistical evaluation of the results is given in Table I. The convulsive states of the 28 subjects investigated may be classified on an etiological basis as follows:

	Cases
Idiopathic	14
Post-traumatic	8
Cortical scar or adhesion (not post-traumatic)	2
" atrophy (degenerative)	4

A comparison of the values of the arithmetical means with those for normal subjects taken from the compilation of Schmidt and Greenberg⁹ is given in Table II. The data show that the levels of

TABLE II.

Comparison of Chemical Composition of Blood and Spinal Fluid in Normal and Epileptic Subjects.

	Plasma Mg	Red Corpuscle Mg	Serum Ca
Normal	2.74±0.3	6.6 ±0.5	10.3 ±0.5
Epilepsy	2.67±0.45	7.72±2.9	10.35±1.3
	Spinal Fluid Ca	Spinal Fluid Mg	Spinal Fluid P
Normal	5.3±0.6	3.3 ±0.2	1.7 ±0.4
Epilepsy	5.3±0.4	2.93±1.15	1.57±0.7

⁶ Greenberg, D. M., and Mackey, M. A., *J. Biol. Chem.*, 1932, **96**, 419.

⁷ Kirk, P. L., and Schmidt, C. L. A., *J. Biol. Chem.*, 1928, **76**, 115.

⁸ Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

⁹ Schmidt, C. L. A., and Greenberg, D. M., *Physiol. Rev.*, 1935, **15**, 297.

the components analyzed are essentially normal with a tendency toward a greater degree of variation than is normally found in red corpuscle magnesium, serum calcium, and spinal fluid magnesium. Such variations from the normal arithmetical means as were found do not appear to be significant. The few instances of abnormally low or abnormally high analytical values that were observed did not fall into any special category of the cases.

If the epilepsy is associated with a disturbance of ion balance, it does not appear to involve magnesium, calcium, or phosphorus.

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Effect of Rapidly Repeated Pregnancies on Transplantable Mammary Rat Adenofibromas.*

LUDWIG A. EMGE AND KATHLEEN M. MURPHY.

From the Department of Obstetrics and Gynecology, Stanford University School of Medicine.

Assuming that rapidly repeated pregnancies stimulate breast tissue to unusual activity and assuming that unusually long storage of milk in such tissue might be detrimental, 20 mature white rats, including 5 males, were simultaneously implanted with a fast-growing rat mammary adenofibroma, 5-B 1. Eleven were bred from 2 to 4 times in close sequence, all producing litters of good size which were immediately destroyed. All tumors were removed at the end of 96 days of growth.

Wide variations were observed in the daily weight gain of tumors in all groups, and no conclusions in regard to weight gain are permissible because of this.

A detailed cytologic study of all tumors showed that the adenomatous tissue underwent hyperplastic changes well within the limit of the ordinary mammary response to pregnancy. Retention of secretion was observed grossly and microscopically in all tumors removed within one week after the last pregnancy. In no tumor was there seen any tendency toward malignant degeneration. In the control groups the tumors of male animals showed a very marked loss of adenomatous components. Those from female controls showed no variations from the donor tumor.

*Aided by grants from the Rockefeller Fluid Research Fund of Stanford University School of Medicine, and from the Scientific Research Committee of the American Medical Association.

Conclusion. Rapidly repeated pregnancy does not affect the adenomatous component of our transplantable rat adenofibroma 5-B 1 beyond that expected to occur in mammary tissue in single pregnancy. Retention of secretory material was observed but no tendency toward malignant hyperplasia was detected.

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**Influence on Eye Color of Feeding Diffusible Substances to
Drosophila melanogaster.***

G. W. BEADLE AND L. W. LAW.† (Introduced by D. M. Whitaker.)

From the School of Biological Sciences, Stanford University.

Diffusible substances of hormone-like nature concerned in eye color development are known in *Drosophila* and in certain other insects. First evidenced in *Drosophila* by observations of interactions between tissues of different constitutions in genetic mosaics,¹ such substances have been studied extensively by means of transplantation and injection experiments.²⁻⁵ The substances designated v^+ substance and cn^+ substance have been obtained in solution and certain of their chemical properties determined.⁶⁻⁸ One test for these substances has been injection of solutions into the blood of test larvae. Presence of active v^+ substance results in a modification of the eye color of vermilion brown ($v\ bw$) animals from a very pale pink toward brown.⁷ A similar modification is produced by cn^+ substance on the eye color of cinnabar brown ($cn\ bw$) test larvae. Experiments have shown that the substances mentioned are taken up effectively when fed to test larvae. It is the purpose of the present paper to describe such experiments.

Preliminary trials showed that if wild type pupae are immersed

* Aided by funds granted by the Rockefeller Foundation.

† Parker Fellow of Harvard University.

1 Sturtevant, A. H., *Proc. Sixth Int. Cong. Genet.*, 1932, **1**, 304.

2 Beadle, G. W., and Ephrussi, B., *Genetics*, 1936, **21**, 225.

3 Ephrussi, B., and Beadle, G. W., *Bull. Biol. Fr. Belg.*, 1937, **71**, 54.

4 Beadle, G. W., Clancy, C. W., and Ephrussi, B., *Proc. Roy. Soc., B*, 1937, **122**, 98.

5 Harnly, M. H., and Ephrussi, B., *Genetics*, 1937, **22**, 393.

6 Khouvine, Y., Ephrussi, B., and Harnly, M. H., *C. R. Acad. Sci., Paris*, 1936, **203**, 1542.

7 Thimann, K. V., and Beadle, G. W., *Proc. Nat. Acad. Sci.*, 1937, **23**, 143.

8 Khouvine, Y., and Ephrussi, B., *C. R. Soc. Biol.*, 1937, **124**, 885.

in boiling water for a short time (30-40 seconds), crushed, and fed on a thin layer of plain agar to vermilion brown or cinnabar brown test larvae taken from regular cultures at about 24 hours before puparium formation, the eye color of both types of test animals is modified toward brown. Unheated pupae, otherwise similarly fed, have no effect. This loss of activity in unheated crushed pupae has been noted previously in injection tests and has been attributed to enzymic oxidation of the substances concerned.⁷

Control experiments show that pupae of genetic types deficient in one or both substances produce no modification in eye color of appropriate test animals. Thus, vermilion pupae fed to vermilion brown test larvae produce no eye color response; similarly, cinnabar pupae give negative tests for *cn*⁺ substance.

In an attempt to standardize and improve the feeding test method, test larvae of known ages were fed at various stages of development. Individual tests were made in the following way: One cc. of 2% agar was pipetted into 25x90 mm. shell vials. Fifty wild type pupae, taken at 48 hours after puparium formation, were heated in boiling water for 30 to 40 seconds, crushed and placed on the agar. Twelve test larvae were placed in the vial. Eggs from which test larvae were obtained were collected over a 2½ hour period and the test larvae were cultured, up to the beginning of the feeding test, on standard cornmeal-molasses-agar medium seeded with yeast. All experiments were carried out at 25°C. The results of a series of such tests are summarized in Table I. It is seen that, under the conditions of the tests, the maximum effects were observed when test larvae were taken for feeding at from 60 to 84 hours after egg-laying. Weaker responses of larvae put in the test vials at an earlier stage may be accounted for by assuming a gradual loss of activity

TABLE I.
Effect of Feeding Crushed Wild Type Pupae on the Eye Color of vermilion brown (*v bw*) and cinnabar brown (*cn bw*).

Age at feeding (hours after egg laying)	<i>v bw</i> test animals		<i>cn bw</i> test animals	
	No.	color value* (mean and range)	No.	color value* (mean and range)
36±1½	12	1.0 (0.7-1.5)	10	0.2 (0.0-0.2)
48±1½	9	0.5 (0.0-0.7)	9	0.1 (0.0-0.2)
60±1½	12	2.1 (1.5-4.0)	12	2.2 (1.5-2.3)
72±1½	10	1.7 (1.5-2.3)	12	1.9 (1.0-2.3)
84±1½	10	1.8 (1.0-2.3)	12	1.7 (1.0-2.3)
96±1½	12	0.1 (0.0-0.3)	12	0.9 (0.0-1.5)
108±1½†	9	0.0	12	0.0

*In the numerical scale of color values, 0 represents no effect, 5 the maximum possible effect.⁷

†Puparium formation took place within a few hours after this time.

of the eye color substances in the crushed pupae. The decline in effect in older test larvae is explained by the fact that they take in less food before puparium formation than do younger larvae.

Tests have shown that somewhat stronger effects are obtained on feeding crushed pupae if the material to be fed is not allowed to come in contact with the agar. The active substances are known to diffuse into agar where presumably they are less available to the larvae.

Active water solutions of the two substances under consideration, prepared and made available by Dr. Edward L. Tatum, have given positive reactions when mixed with yeast and fed as described above.

Summary. The hormone-like v^+ and cn^+ substances concerned in the development of eye colors in *Drosophila* produce their characteristic reactions when administered with the food of test larvae. In this way they are comparable with certain hormones of the vertebrates, for example, thyroxin.

9666

Electrophoresis of Prolan.*

M. A. SPIELMAN AND ROLAND K. MEYER.

From the Departments of Chemistry and Zoology, University of Wisconsin.

No gonadotropic substance has ever been isolated in pure form. Nevertheless, an empirical idea of the chemical nature of these substances has been deduced from their solubility, their failure to diffuse through membranes, and their inactivation by certain reagents. The gist of these experiments¹⁻⁴ is that the gonadotropic hormones are protein-like in character and perhaps are muco-proteins similar to certain enzymes.

The object of the present work is to bring a new technique, namely that of electrophoresis, to bear upon the problem of the characterization of gonadotropic substances. The behavior of an amphoteric substance such as a protein in an electrophoretic cell

* Supported in part by a grant from the Wisconsin Alumni Research Foundation.

¹ Fischer, F. G., and Ertel, L., *Z. physiol. Chem.*, 1931, **202**, 83.

² Hanrowitz, F., Reiss, M., and Balint, J., *Ibid.*, 1933, **222**, 46.

³ Biscoff and Long, *J. Biol. Chem.*, 1936, **116**, 285.

⁴ V. Euler, H., and Zondek, B., *Chem. Abs.*, 1934, **28**, 4085; *Skand. Arch. Physiol.*, 1934, **68**, 232.

can, in favorable cases, reveal the isoelectric point of the compound. This is probably the only definite, measurable property of a protein which may be determined by physical means in a crude mixture.

Prolan, the gonadotropic principle of pregnancy urine, was selected as the first of a series of substances to be investigated. A sample of so-called alcohol prolan, obtained by precipitation with alcohol, was used as a preparation least likely to have undergone chemical change in isolation. A second preparation was made by the brucine method of Katzman and Doisy.⁵

Crude alcohol prolan was prepared from late pregnancy urine by precipitation with 18 volumes of alcohol. The dried powder was assayed by the method described below. Twenty milligrams gave ovaries weighing 40 mg. Brucine prolan prepared by Katzman and Doisy's method,⁵ was similarly assayed; 16 mg. gave 24 mg. ovaries.

The electrophoresis apparatus was of the conventional U-type.⁶ The central chamber was of 12 mm. bore and held 20 cc. The adjoining anode and cathode chambers held 10 cc. each. Electrodes were of sheet copper and set into 20 cc. cups sealed to the bottom of 250 cc. flasks. The anode was packed with rock salt and the cathode with crystals of copper sulfate. The current source was the 110 volt D.C. line without resistance.

Alcohol Prolan. Four hundred milligrams was dissolved in 20 cc. of water, and 5 cc. of molar sodium acetate-acetic acid buffer solution was added. The material became completely soluble in a few minutes. The pH was measured with a quinhydrone electrode. It was placed in the central chamber, and after rinsing, the anode and cathode chambers were filled with M/15 buffer solution of the same pH, and the electrophoresis was begun. After 18 hours the anodic and cathodic fractions were collected separately and neutralized. The pH of the central chamber was checked, and in no case was the drift more than 0.08 of a pH unit. Mean pH values are given in Table I. The procedure was repeated with fresh material and the 2 samples were pooled.

Four rats each were used for assaying the contents of the cathode and of the anode chambers at each pH value. The animals were immature female rats, 21 days old at the time the injections were begun. Each rat received a total quantity of 4.5 cc., 0.5 cc. of this being injected twice daily for 4.5 days, with autopsy on the morning of the sixth day.

⁵ Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1934, **107**, 513.

⁶ Hartman, R. J., and Cheng, L. T., *J. Phys. Chem.*, 1936, **40**, 454.

TABLE I.
Electrophoresis of Alcohol Prolan.

pH	Anode			Cathode		
	Foll.	C.L.	Ut. Stim.	Foll.	C.L.	Ut. Stim.
4.91	4*	4	4	0*	0	0
4.57	4	2	4	0	0	0
3.95	3	2	4	0	0	0
3.70	3	0	4	0	0	0
3.55	1	0	2	0	0	0
3.31	0	0	0	0	0	0

*Figures indicate the number of rats showing follicular development, corpora lutea, or enlarged uterus.

The presence or absence of gonadotropic material in the anode, cathode, and in certain cases in the central chamber was determined by the ability of samples of the contents to cause development of follicles, corpora lutea, or the uterus. The weight of the ovaries of all animals showing stimulation of one or more of these structures was not much greater than that found for untreated immature animals of the same age. In Table I are recorded the number of animals showing development of corpora lutea, follicles or uterus. No attempt was made to make a study of the precise quantity of gonadotropic material which migrated from the central chamber.

Brucine Prolan. One hundred milligrams were used in each charge. Otherwise the procedure was as before. The data are recorded in Table II.

TABLE II.
Electrophoresis of Brucine Prolan.

pH	Anode			Cathode		
	Foll.	C.L.	Ut. Stim.	Foll.	C.L.	Ut. Stim.
5.8	4*	4	4	1*	0	0
5.1	4	3	4	3	1	3
4.4	4	3	4	4	2	4
4.0	4	4	4	4	4	4
3.5	4	1	4	4	1	4

*Figures indicate the number of rats showing follicular development, corpora lutea, or enlarged uterus.

The data of Table I show that with alcohol-precipitated prolan there is definite and unmistakable gonadotropic activity in the *anodic* material from pH 4.91 to pH 3.55, with a decrease in activity toward the acid side. At no pH does the hormone behave as a cation. At pH 3.55 where migration is slight, an appropriate blank experiment and analysis of the central chamber showed that the absence of activity is not attributable to acidity or to heating by the electric current. This would suggest an approach to the iso-

electric point, but as the table indicates, increase of acidity to pH 3.31 did not induce migration to the cathode although bioassay of the contents of the central chamber demonstrated considerable, but a lesser than expected amount of gonadotropic activity. This indicates that the high acidity probably is responsible for the absence of activity in the anode and cathode chambers at pH 3.31. Prolan is definitely inactivated in greater concentrations of acid. The data indicate that alcohol prolان has an isoelectric point on the acid side of pH 3.5, and probably in the neighborhood of pH 3.3. Isoelectric points in this highly acid range are characteristic of mucoproteins.⁷

An entirely different set of results was obtained from prolان prepared by the brucine technique as shown in Table II. Such material was necessarily subjected to the action of strong acids and bases, and hence had probably undergone chemical change. Gonadotropic activity was found in both *anode* and *cathode chambers* from pH 5.1 to 3.5. The data suggest that the prolان complex consists of a non-specific carrier in combination with a specific residue which continues to be biologically active after the carrier has undergone rather profound change as evidenced by shift of isoelectric point. A similar hypothesis has been advanced by Euler and Zondek,⁴ and comparable electrophoretic behavior has been observed with oxytocin.⁸

Concerning the problem of the plural nature of gonadotropic hormones, we are prepared to make no allegations on the basis of our experiments. We have observed exclusive follicular development induced by specimens obtained at those pH values whereat migration was slight, but this is the usual phenomenon at low dose levels.

Summary. Alcohol prolان shows the electrokinetic behavior of a single chemical individual. The isoelectric point is more acid than pH 3.5. Brucine prolان behaves as a mixture. The physiological activity of prolان is attributed to a grouping which may survive chemical treatment drastic enough to alter the protein-like carrier.

⁷ Roche, A., *Compt. rend. soc. biol.*, 1935, **120**, 1229.

⁸ Das, N., Ghosh, B. N., Guha, B. D., *Z. physiol. Chem.*, 1936, **238**, 131.

9667 P

Circulatory Effects of Pentothal Sodium.

CHAPMAN REYNOLDS AND J. ROSS VEAL.

*From the Departments of Pharmacology and Surgery, School of Medicine,
Louisiana State University, New Orleans.*

Many of the early reports of the action of pentothal sodium indicated relatively little effect on heart and circulation as compared with the more conspicuous respiratory depression.¹ Since with most anesthetics it is usually not difficult to restore respiration if the circulatory apparatus is relatively intact, this drug would seem to be fairly safe when used with discretion.

Curiosity about the effects of overdoses and of repeated doses prompted the introductory experiments here briefly reported. The work was done on 12 dogs and 10 cats. No ether or other general preliminary anesthetic was used: carotid, tracheal and femoral canulae being introduced after careful procaine local analgesia and gentle operative procedure.

Minimal narcotic doses (0.020 per kg. intravenously) slowly given ordinarily do not arrest respiration or lower the blood pressure. The same dose given too rapidly may cause a cessation of breathing without any measurable effect on blood pressure. When a larger sublethal dose (0.025 per kg.) is administered there is usually an arrest of respiration, sometimes an appearance of cardiac irregularity, but without an essential fall in blood pressure. These mild effects usually disappear spontaneously, or more rapidly with the aid of artificial respiration. After restoration of breathing, no further deleterious effect on respiration or blood pressure appears.

In one instance a dog, after repeated doses, was "resuscitated" 18 times in the course of an hour and 50 minutes by artificial respiration. Eventually blood pressure dropped suddenly to 0, and we were unable to restore it.

When, however, the original dose (0.020 kg.) was repeated after the return of reflexes, there was not only a longer period of narcosis, but respiration was arrested for a longer time, there was a more pronounced heart arrhythmia and sometimes a slight fall in blood pressure. After return of reflexes, repeated doses of the same size have a more pronounced effect on the blood pressure curve, showing

¹ Lundy and Tovell, *Proc. Mayo Clinic*, 1935, **10**, 557, 743, 791; Pratt, Tatum, et al., *Am. J. Surg.*, 1936, **31**, 464; Gruhzit, Dox, et al., *J. Pharm. and Exp. Therap.*, 1937, **60**, 125.

definitely the slow, deep rhythm described by Gruber,² and ascribed by him after electrocardiographic studies to the development of ventricular ectopic beats interspersed with long compensatory pauses.

After the administration of a minimal narcotic dose, 4 of the dogs were carried along as nearly as possible at the level of abolition of corneal and joint reflexes by saline-diluted fractional doses (0.003-0.005) of pentothal sodium as indicated. There was a progressively longer narcotic effect after each successive administration. In each case, death followed an unwarned sudden drop of blood pressure to the base line, sometimes before respiration had ceased. The suddenness of the blood pressure fall and the previous appearance of ectopic ventricular contractions suggest the development of ventricular fibrillation.

It is of interest that in cats with 2 or 3 repeated minimal anesthetic doses intraperitoneally, respiration continued for as long as 2 to 5 days before the unconscious animals finally died.

Summary. The first undesirable effect of a single sublethal dose of pentothal sodium is sudden cessation of respiration. Repeated administration of small amounts, sufficient to maintain light anesthesia, suddenly produces signs of heart muscle poisoning. There seems to be a definite cumulative action. Whether this is due to damage of liver tissue, or whether the drug is changed into simpler, larger dose, and longer acting forms (diethyl-barbiturate?) we are not prepared to suggest. The longer the administration is continued, the more does the threat seem to shift to the more fatal circulatory side.

9668 P

Bleeding Tendency of Obstructive Jaundice: Prothrombin Deficiency and Dietary Factors.*

E. D. WARNER, K. M. BRINKHOUS AND H. P. SMITH.

From the Department of Pathology, State University of Iowa.

Hawkins and Whipple¹ have described in bile fistula dogs a bleeding tendency which is prevented by feeding whole bile. Hawkins and Brinkhous² showed that the bleeding is due to a prothrom-

² Gruber, Charles M., *J. Pharm. and Exp. Therap.*, 1937, **60**, 143.

* Aided by a grant from the Committee on Scientific Research of the American Medical Association, and more recently by a grant from the John and Mary R. Markle Foundation.

¹ Hawkins, W. B., and Whipple, G. H., *J. Exp. Med.*, 1935, **62**, 599.

² Hawkins, W. B., and Brinkhous, K. M., *J. Exp. Med.*, 1936, **63**, 795.

bin deficiency. That low prothrombin values may occur in obstructive jaundice has been suggested by Quick, Stanley-Brown and Bancroft.³ We wish to report our experience with human cases of obstructive jaundice and of biliary fistula.

The prothrombin titration method is one which we have described previously.⁴ Many of our jaundiced cases showed no bleeding tendency, and in them the prothrombin level was usually above 50% of normal. If the prothrombin level is below 35% one often encounters bleeding from mucous membranes or hemorrhage at operation or from the wound afterward. A subsequent rise in the prothrombin level is associated typically with cessation of bleeding. In 2 cases, for example, spontaneous hemorrhage occurred with prothrombin values of 33 and 16% respectively. Recovery from the hemorrhagic tendency in these was associated with a prothrombin rise to 92 and 66%.

The restoration of bile to the intestinal tract results in a gradual rise in the prothrombin level. This is shown in cases where the obstruction is suddenly released, or in cases where bile is fed by mouth. In one case the patient was bleeding, with a prothrombin level of 10%. Then there was spontaneous and practically complete relief of the obstruction and the bleeding tendency ceased a few days later. At the end of 2 weeks the prothrombin had risen to the 89% level. In another case in which there was a biliary fistula of 4 months' duration, bleeding from a recent operative incision occurred. The prothrombin level was 27% of normal. The bile which drained from the fistula was then fed by mouth. With this therapy the prothrombin gradually rose to 89% of normal over a period of 7 weeks. Bleeding ceased after the prothrombin had risen above the 30% level.

These patients undoubtedly suffer from disturbances in the digestion and absorption of fats. This difficulty is probably overcome, at least in part, by restoration of bile to the intestinal tract. In "hemorrhagic chick disease," due to a deficiency in the fat-soluble anti-hemorrhagic vitamin, low prothrombin values have been reported.^{5, 6} Alfalfa, as shown by Almquist and Stokstad,⁷ is rich in this vitamin. We have followed the prothrombin level in human cases in

³ Quick, A. J., Stanley-Brown, M., and Bancroft, F. W., *Am. J. Med. Sc.*, 1935, **190**, 501.

⁴ Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.

⁵ Dam, H., Schönheyder, F., and Tage-Hansen, E., *Biochem. J.*, 1936, **30**, 1075.

⁶ Quick, A. J., *Am. J. Physiol.*, 1937, **118**, 260.

⁷ Almquist, H. J., and Stokstad, E. L. R., *J. Biol. Chem.*, 1935, **111**, 105.

which bile feeding was supplemented with a fat soluble extract of alfalfa meal. The residue of a petroleum ether ("Skellysolve A") extract of 200-400 gm. alfalfa meal was emulsified in human bile, or, in some cases, in 2% sodium taurocholate solution, and fed daily. In a number of patients given this alfalfa-bile mixture, the rise in prothrombin was very rapid, even with persistence of jaundice. If bleeding had existed, it soon ceased. In 3 cases of obstructive jaundice, the prothrombin level before therapy was 43, 20, and 37%. Alfalfa with bile or bile salts was given for 6, 8, and 6 days respectively. At the end of the period, the prothrombin values had risen to 86, 107, and 102%. The rise in these cases is considerably faster than with simple bile feeding.

In these cases, whole blood clotting time and Duke's bleeding time were usually within normal limits despite considerable reduction in plasma prothrombin. Significant prolongations were found only when the prothrombin was at very low levels and bleeding was imminent or actually present.

The data presented show that the bleeding tendency in cases of obstructive jaundice and of biliary fistula is due to a low plasma prothrombin level. The importance of bile in the intestinal tract is shown by the return of the prothrombin to normal levels when bile is restored to the intestine. The fact that the prothrombin rises with greater speed when an alfalfa extract is fed along with bile suggests that Vitamin K is concerned. However, this vitamin deficiency disease has not been produced in normal mammals by feeding deficient diets.

Summary. 1. The bleeding tendency in cases of obstructive jaundice and biliary fistula is due to a prothrombin deficiency. 2. With restoration of bile to the intestine the prothrombin gradually returns to normal and the bleeding ceases. 3. Feeding of alfalfa extract, rich in Vitamin K, in addition to bile, causes a more rapid return of the prothrombin to normal.

9669

Locus and Physiology of Photoperiodic Perception in Plants.

W. F. LOEHWING.

From the Department of Botany, State University of Iowa.

Photoperiodic responses of intact plants whose bases and tops have been exposed to contrasted length of daylight, indicate that the stimulus is not normally transmitted from the exposed area.¹ Flowering is usually restricted to the zone receiving the reproductive photoperiod. More recently, however, by the use of reciprocal grafts of photoperiodically sensitized plants, it has become possible to demonstrate stimulus transfer from one part to another across the graft union.² Opaque boxes and cloth bags have also been employed in studies on the interrelationships of contrasted photoperiods on adjacent parts.^{1, 3}

The graft technique is not wholly satisfactory in studies of this sort, however, because it is difficult, entails delay and post-operative care, causes considerable mortality and involves the extraneous stimulus of severe traumatism. The results of whip, cleft and bud grafts vary greatly.^{4, 5} The use of small bags or boxes impairs gas exchange and creates temperature differences between the exposed and shaded regions, thereby introducing possibility of error since the developmental temperatures of apical meristems also influence the reproductive behavior of many plants. Bagging is tedious and apt to involve injury whenever a large number of multistemmed plants is employed.

In order to minimize these difficulties and at the same time employ contrasted photoperiodic exposure on contiguous parts, plants were grown to a convenient height under vegetative light conditions on opposite sides of a thin, opaque panel having a long, 2-inch horizontal slit. Tops of plants were trained through the slit to various desired lengths from both sides of the panel, and one side was then given long day and the other short day illumination. Long photoperiods were provided by use of automatically controlled electric lights hung from pulleys. Short day exposure was obtained by use of adjustable, black curtains suspended from a light-weight frame

¹ Garner, W., and Allard, H., *J. Agric. Res.*, 1925, **31**, 555.

² Kuijper, J., and Wiersum, L. K., *Proc. Acad. Sci. Amsterdam*, 1936, **39**, 1114.

³ Rasumov, V. J., *Planta*, 1935, **23**, 384.

⁴ Cajlachjan, M. C., and Yarkovaja, L. M., *Comp. Rend. Acad. Sci. URSS*, 1937, **15**, 215.

⁵ Moskov, B. S., *Comp. Rend. Acad. Sci. URSS*, 1937, **15**, 211.

on the panel. In this way, the tops of the plants on one side received long day and their bases short day photoperiods, while reverse illumination of parts prevailed for plants on the opposite side of the panel. This arrangement made it possible to interchange the photoperiodic stimulus on the 2 sides without moving the plants.

Seeds of Ito San soybean, a variety flowering in short day, were sown in fertilized soil in 2 gallon jars, 4 plants per jar and 30 jars on each side. A 14-hour day was employed on both sides until plants were 14 inches tall and then a 9-hour day was initiated on one side by use of automatically controlled electric lights. Tops are uniformly designated as "a" in each group, and bases of the same plant receiving the opposite light treatment as "b" (Table I). Plants were divided into 4 groups of 30 on each side of the panel as follows: first and second with the entire upper half of fully foliated shoots protruding through the panel (Groups 1 and 2, Table I). The third and fourth groups were similar except that the protruding upper half of shoots was defoliated except near the tip (3 and 4, Table I). The short day bases of this group were kept continuously exflorated (4b, Table I). The fifth and sixth groups were like the first except that their bases were kept defoliated (5b, 6b) and the short day tops exflorated (5a). In the seventh and eighth groups only 2 inches of the apices projected through the slit (7 and 8).

TABLE I.
Effects of Contrasting Photoperiods on Tops and Bases of Single Plants of Ito San Soybean, a Variety Normally Flowering in Short Day.

Group	Part of Plant	Day Length, in Hours	Other Treatment	Plants in Flower, %	Age at Flowering, days
1a	Top	9	None	100	38
1b	Base	14	"	0	—
2a	Top	14	"	0	—
2b	Base	9	"	100	38
3a	Top	9	Defoliated	0	—
3b	Base	14	None	0	—
4a	Top	14	Defoliated	80	45
4b	Base	9	Exflorated	0	—
5a	Top	9	Exflorated	0	—
5b	Base	14	Defoliated	87	42
6a	Top	14	None	0	—
6b	Base	9	Defoliated	0	—
7a	Apex	9	None	0	—
7b	Base	14	"	0	—
8a	Apex	14	"	0	—
8b	Base	9	"	100	38

Ten days after start of short-day lighting, flowers began to appear on this side (1a, 2b, 8b, Table I). Four days later, flowers appeared in the fifth group, *i. e.*, on long-day, defoliated bases (5b) of plants whose short-day tops had been kept defoliated, followed in another 3 days by flowers on the long day, defoliated tops of the exfoliated short-day bases in the fourth group (4a). Thus, it is necessary to defoliate a portion of the dually lighted shoots of soybean to demonstrate the possibility and actual occurrence of transfer of the flowering stimulus. That flowers formed on the defoliated shoots in long day (4a) are the result of the short-day stimulus on the opposite side of the panel is shown by the sequence of flower development. Flowers form first nearest the point of reproductive stimulus, namely nearest the panel and the foliage receiving short day. The flowering stimulus is conveyed downward more readily than upward if the delay in flowering of the defoliated shoots be taken as a criterion. The exfoliated bases (5b) flowered 3 days sooner than the corresponding exfoliated tops (4a).

Defoliated short-day tops (3a) and bases (6b) do not flower, a response which indicates that in soybean the leaves are the perceptive zones of the light stimulus. Though it might seem that failure of reproduction of the defoliated tops (3a) and bases (6b) was due to lack of food, this explanation is precluded both by the healthy appearance of the plants and the fact that flowers did form on the defoliated long-day tops (4a) and bases of exfoliated series (5b). Short-day illumination of apex alone (7a) was inadequate for flower formation.

From the foregoing it is evident that the slit panel technique offers a simple and rapid method of subjecting different parts of the same plant to contrasted photoperiods. It permits ready use of larger populations than have been previously reported in studies of this sort, and this with a minimum of injury and care.

The responses to differential lighting are clear cut. The data, considered in conjunction with those of the workers cited and the established fact that photoperiodic responses can be induced in light intensities far below the threshold of photosynthesis, indicate that the flowering stimulus is a foliar influence entirely distinct from carbohydrate synthesis. In fact, the formative floral organization of the plastic nutrients synthesized in leaves apparently depends upon one or more specific inductors whose production in the soybean results from short-day illumination. The stimulus is obviously due to a hormonal or quasi-catalytic substance of foliar origin, normally inducing reproduction in closely adjacent areas in

such a way that its translocatability is ordinarily not conspicuous in intact plants. Yet by properly combining the effects of exfloration and defoliation with photoperiodic illumination, it is possible to demonstrate not only stimulus transfer, but flower inception in normally vegetative regions.

The vegetative stimulus of long-day also appears to exert an inhibitive influence upon stimulus transfer because, in intact plants flowering is restricted to regions receiving short-day treatment, whereas in the defoliated plants (4a and 5b), on the other hand, there is clear cut evidence of transfer. In fact, both the flowering stimulus of short-day and its inhibition in long-day exhibited a direct quantitative relationship to the amount of foliage. The largest number of flowers on defoliated parts appeared on those plants with the greatest number of leaves under short-day illumination. Contrariwise, impedance of transfer varied directly with the number and size achieved by leaves before their removal from defoliated parts.

The postulation of a specific florigenic inductor derives further support from the recent conclusive determination that carbohydrate-nitrogen relations in the soybean are not, as hitherto frequently assumed, the direct causative factors of flowering.⁶ Formation of flower primordia precedes the shift in nutrient balance characteristically associated with the transition from the vegetative to the reproductive phase. It seems safe to predict the early isolation and identification of this florigenic agent, a discovery which may provide an explanation for the paradox of its formation in some species under long-day and in others under short-day conditions.

9670

A Stable Metaphosphate Preparation for Use as a Protein Precipitant.*

DAVID R. BRIGGS. (Introduced by R. A. Gortner.)

From the Department of Biochemistry, University of Minnesota.

That metaphosphoric acid causes essentially quantitative precipitation of proteins, in pH ranges acid to the isoelectric point of

⁶ Murneek, A. E., *Mo. Agric. Exp. Sta. Res. Bul.*, 1937, **268**, 84.

* Contribution from O. S. A. Sprague Memorial Institute, University of Chicago, and Division of Agr. Biochemistry, University of Minnesota. Paper No. 1550, Journal Series, Minnesota Agricultural Experiment Station.

the protein, has long been known. Its use as a means of removal of the protein from blood serum preliminary to analysis for other components of the blood has been studied by Folin¹ and by Hiller and Van Slyke.² Due to its instability in aqueous solution and to the difficulty of obtaining a uniform commercial reagent, metaphosphoric acid has lost favor as a standard protein precipitant, although its action closely simulates that of the more expensive trichloroacetic acid commonly used (in 10% solution).

The object of this paper is to describe a method of preparation and manner of use of metaphosphoric acid which will largely eliminate the objections which have tended to prevent its use as a routine protein precipitant.

Metaphosphoric acid is commonly prepared by heating the ortho-acid to drive off one mole of water per mole of ortho phosphoric acid, or by the addition of one mole of water to one mole of P_2O_5 . The acid so formed is a strong acid ($pK_a = \text{ca. } 1.5$) and may be highly polymerized. When placed in solution in water, it is hydrolysed rapidly back to the ortho-acid. The commercial meta acid, obtained as sticks of glacial metaphosphoric acid, always contains sodium, usually containing approximately one atom of Na per 2 atoms of P. It is sometimes given the formula, $NaPO_3 \cdot HPO_3$. Titration curves made on solutions of such products, however, show that all of the acid which is present is in the ortho form. The remainder of the phosphorus is normally present as sodium meta phosphate. When a solution of the "glacial" acid is boiled, all the meta phosphate present is changed quickly to ortho, the same reaction being accomplished at room temperature in the course of 2-3 months. The hydrogen ion is the catalyst for this hydrolysis of the meta to the ortho form, and its action is enormously intensified by heat. If the solution is brought to a pH of 7 shortly after preparation, the meta phosphate is stabilized and will be hydrolyzed only very slowly even at the boiling temperature of the solution (as determined by titration of the second hydrogen of the ortho phosphate present).

The method of preparation and use of meta phosphoric acid given below is based upon the evidence that, in solution, meta phosphoric acid itself is very unstable while the sodium salt of meta phosphoric acid, especially in a solution made somewhat alkaline with Na_2CO_3 or NaOH, is stable.

The method recommended for the preparation of the stable Na

¹ Folin, O., *J. Biol. Chem.*, 1919, **39**, 259.

² Hiller, A., and Van Slyke, D. D., *J. Biol. Chem.*, 1922, **53**, 253.

metaphosphate is that of heating $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ to drive off 2 molecules of the H_2O . When this is done, it is found that at a relatively low temperature, the one water of crystallization is eliminated and that by the time the temperature has reached 400°C . a second water has been completely lost. At this point, the preparation, which has the formula $(\text{NaPO}_3)_x$, has the appearance of a white amorphous powder soluble in water. This salt shows all of the precipitations characteristic of pyrophosphates, but has the ability to precipitate protein in acid solution (which is a characteristic test for meta phosphate to distinguish it from pyrophosphate and orthophosphate). If now, the white powder $(\text{NaPO}_3)_x$ is further heated to about 700°C . it melts and forms a glass upon cooling. Solutions of this form of meta phosphate have a greater power to precipitate protein (do so in lower concentrations) than do those obtained from the amorphous powder prepared at 400°C . Conductivity-concentration curves and freezing point depressions measurements made with solutions of these 2 forms of Na metaphosphate show³ that the white powdery Na metaphosphate acts as a uni-trivalent strong electrolyte, having a formula $\text{Na}_3(\text{PO}_3)_3$, while the glass form acts as a weak electrolyte so highly polymerized that, except at very high dilutions, only a part of the Na is ionized. The valence of the complex polymerized ion is much higher than 3. No formula can be given for this form of Na meta phosphate, at present. Both of these forms are relatively stable in water solution and retain their power to precipitate protein (in acid solution) over a period of months after standing at room temperature. However, hydrolysis occurs slowly and it is possible that depolymerization proceeds also over that period of time. The latter phenomenon is being further studied, but it seems to be a rather slow process, if it occurs at all separate from the process of hydrolysis. Due, perhaps, to traces of NaH_2PO_4 which will remain even after long periods of heating the melted salt, the pH of aqueous solutions of these preparations of Na metaphosphate is of the order of 5.5. If a small amount of Na_2CO_3 or NaOH is added to the solutions of these salts, bringing the pH to 8 or 9, the stability of the metaphosphate, at ordinary temperatures, is practically permanent. This is illustrated in Table I, where the stability, at the boiling temperature, is measured at the various pH values.

The amount of hydrolysis was measured by titrating the second hydrogen of the ortho phosphate present at the end of the time of boiling the solutions. The solution containing equimolar quanti-

³ Briggs, D. R., paper in preparation.

TABLE I.

% of Hydrolysis of Sodium Metaphosphate (glass) in 2% Solution After Boiling the Solutions at Various Initial pH Values for Specified Lengths of Time.

Solution	Time of Boiling, hr.	Approximate % Hydrolyzed
Freshly dissolved Na metaphosphate	0	0.5
„ „ „ „ „ (pH = 5.5)	1	10.0
Na metaphosphate and NaOH to pH = 7	1	2.0
„ „ „ „ „ pH = 9	1	0.6
„ „ „ „ „ pH = 10	1	0.5
1 mole Na metaphosphate + 1 mole H ₃ PO ₄ pH = 2	½	100.0

ties of the Na metaphosphate and ortho phosphoric acid corresponds to a solution of commercial glacial metaphosphoric acid.

A method of preparing sodium meta phosphate (glass) is as follows: Pure $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ is heated, carefully at first to prevent spattering, and then strongly in a muffle furnace or over a gas flame until the whole of the salt is melted into a clear melt. It should be maintained at this temperature for perhaps an hour to insure complete expulsion of water. The heating may be done in a porcelain crucible. Slight pitting of the glass covering of the crucible will occur, however, and a platinum crucible is better. The melt, when cooled to the point where it will just flow should be poured on a platinum surface or removed from the crucible in drops on a platinum wire. (Other metal surfaces may be used if traces of salts of these metals are no detriment in subsequent use of the meta phosphate.) After cooling, it will easily separate from the metal and can be kept dry indefinitely in this form. A 10% solution may be made up and sufficient NaOH or Na_2CO_3 added to bring the pH of the solution to 8 or 9. This may be kept at room temperature. It will maintain its original precipitating efficiency for at least several months.

When using this solution to precipitate proteins an amount of metaphosphate solution equivalent to 0.2 gm. of sodium metaphosphate per gm. of protein constitutes an adequate excess. Thus to 100 cc. of blood serum containing approximately 8 gm. of protein 16 cc. of 10% solution would be added. Sufficient acid, HCl or HAc, is then added to bring the solution to a pH of 2.5 or 3.0, under which conditions the protein will be quantitatively removed as a flocculent white precipitate.

As a protein precipitant, metaphosphoric acid has an advantage over trichloroacetic acid in that it is so much cheaper. Also, such high concentrations of precipitant are not necessary to cause complete removal of the protein from solution when metaphosphoric

acid is used. It can be completely removed by dialysis against water at pH of 7 or 8. It does not precipitate amino acids and is no stronger a precipitant for polypeptides than is trichloroacetic. The above method of preparation and use eliminates its usual disadvantage of instability and variability in precipitating power.

9671 P

Determination of Iodine in 5 cc. of Blood.

J. F. McCLENDON AND A. CALVIN BRATTON. (With the technical assistance of Ralph White and William Foster.)

From the Laboratory of Physiological Chemistry, University of Minnesota.

Four and one-half inches of $\frac{3}{8}$ " Visking sausage casing is moistened at one end and tied to a frame. Five cc. of blood is run into the open end from a syringe or pipette. The open end is tied and the string attached to the other side of the frame, stretching the casing as much as it will bear, and it is dried 40 min. at 100° in an oven. It is then placed in a screw-feed stoking device with detachable water-cooled platinum tip and burned with 120 cc. of oxygen per minute in a platinum combustion tube heated by gas. The screw feed is advanced 1" in 20 min. by motor. The combustion products are led through a pyrex absorber containing 3 sintered glass discs and 25 mg. of sodium azide in 10 cc. of water. At the end of the combustion the stoking tube is removed from the platinum tip, which is allowed gradually to heat, the gas flame is turned off, and the absorber contents and ash are transferred to a 100 cc. pyrex still, using 4 cc. $N/10$ NaOH and 10 cc. H_2O to rinse out. Most of the water is evaporated off while passing a small current of air to prevent bumping, and 3 cc. $6 N H_2SO_4$ is introduced into the still. The iodine is distilled over into a 10 cc. receiver containing 1 cc. of bromine water without immersing the tip. The trap is flamed to prevent iodine remaining in drops of water in it. When SO_3 fumes appear in the still, the flame is reduced to 5 mm. and continued for 4 more minutes. After allowing the air to continue until all condensate has run down into the receiver, the tip is washed, volume made to 9 cc., and an air current blown through it in a steam bath for $\frac{1}{2}$ hour to remove the bromine. Twelve and one-half mg. KI is introduced, the volume is made to 10 cc., and it is titrated with 0.001 N sodium thiosulfate in a Lochte-Hoover burette with divi-

sions of 0.001 cc. (while rapidly stirred) using an electrometric method for indicating the end-point. Burette readings are plotted against voltage, the curve showing a sharp angle at the end-point. Blanks are run on all the reagents and subtracted from the results. Human blood gave 0.1-0.7 γ in 5 cc. On adding iodine, the following was recovered (Table I):

TABLE I.
Micrograms of Iodine in 5 cc.

Original content	Amount added	Amount recovered	% recovered
.5	5	4.81	88
.5	5	4.91	89
.5	5	5.33	97
.5	5	5.51	100

9672 P

A Simple Inexpensive Method for Concentrating Serum Under Sterile Conditions.

WILLIAM THALHIMER.

From Manhattan Convalescent Serum Laboratory, New York City.

Cellophane in sheets has been used for some time as a dializing membrane, and bags made of these sheets have been used as dializing bags.

Seamless tubes of cellophane, known commercially as sausage casings, also have been used for similar purposes.

Schwartzman¹ has made use of cellophane bags in the "preparation and preservation of immunologically active bacterial products."

Kendall² has used cellophane tubes for concentrating antisera and antibody solutions with the aid of a very interesting, simple negative pressure dialysis apparatus which he has devised. Undoubtedly others have used cellophane for similar purposes.

Apparently it is not well known that cellophane can be sterilized in an autoclave at 15 pounds pressure for 30 minutes without changing any of its important characteristics.

It is very simple to make up cellophane bags out of cellophane tubing*—simply tied off with heavy twine at one end and with a

¹ Morell, Sam, and Schwartzman, Gregory, *Science*, 1937, **86**, 130.

² Kendall, Forrest E., *J. Exp. Med.*, 1930, **51**, 319.

*Cellophane sausage casings were generously furnished by Visking Casing Corp.

one-hole rubber stopper tied in at the other end. Through the hole in the stopper a piece of glass tubing is inserted and its end covered with filter paper. The completed bag is sterilized in the autoclave. After it has cooled, sterile, filtered serum is introduced and the bag suspended in front of an electric fan in a room or in a mechanical refrigerator. It has been found that moisture will evaporate from a large cellophane tube filled with serum (about 1,000 cc.) in front of the fan at room temperature at the rate of 20-25 cc. an hour, and almost this fast in the refrigerator. In small amounts, 1-20 cc., in small bags, the serum will concentrate or dry even more quickly.

The serum can be concentrated to any desired degree or can be completely dried. Since the cellophane is impervious to bacteria the serum will remain sterile. When the desired concentration has been reached the serum either can be used or put into sterile bottles and kept. During the process of evaporation, because of the cooling effect of this process, this serum is kept at a temperature about 8°C. below a room temperature of about 25°C.

Serum concentrated to one-fourth the original volume is quite viscous, but can be drawn through a large bore needle for subcutaneous or intravenous injection.

My interest is in concentrating human serums, such as immune measles serum obtained from adults who some years previously had an attack of measles. This concentrated serum is needed to amplify the supply of measles convalescent serum for the prevention or attenuation of measles in children exposed to this disease.

Apparently there also is a growing field of usefulness for concentrated normal human serum for intravenous administration to counteract shock, for the alleviation of edema of individuals with the nephritic syndrome, etc.

The cost of the cellophane tubes is very small and no additional apparatus is needed, so that the additional expense of concentrating serum is very small. However, for concentrating large volumes of serum, instead of rubber stoppers, we are now using duraluminum metal parts, made for us by a machinist.†

If desired, the serum can be evaporated to complete dryness and when dry has the appearance of amber or resin. This method, because of its simplicity and low cost, should be of use in research laboratories for drying and preserving small amounts of serums.

The keeping quality of several standardized serums, dried by this method is now being investigated.

The dried serum can be restored to its fluid condition by merely

† Mr. Paul Leo, 119 West 63rd Street, New York City.

immersing the cellophane bag containing the serum in a vessel of distilled water or physiological saline solution, water dialyzing into the bag. Since bacteria will not pass through the intact cellophane membrane the redissolved serum will remain sterile even though the water in which the bag is immersed is not sterile. The residue of a small amount of serum, 10-20 cc., will redissolve under these conditions in from 1-2 hours.

9673 P

Experimental Exchange Transfusions for Reducing Azotemia. Use of Artificial Kidney for This Purpose.

WILLIAM THALHIMER.

From the Manhattan Convalescent Serum Laboratory, New York City.

Howell¹ first isolated heparin, which retards the coagulation of blood whether added *in vitro* or *in vivo*. Purified, non-toxic heparin was prepared by Charles and Scott.^{2, 3, 4} This preparation has been demonstrated by Best^{5, 6, 7} and his co-workers to be non-toxic when injected intravenously into humans.

A new type of transfusion, called for the time being, an "exchange transfusion," has been carried out experimentally by using heparin* as a non-coagulant.

After removing both kidneys from a dog the animal's blood urea increases rapidly. The morning after bilateral nephrectomy this animal and a donor animal are anesthetized by nembutal† injected intravenously. Cannulae are inserted into the femoral artery and vein of one leg of each animal. At this time a small amount of heparin was injected intravenously into each animal. With a 50 cc. all-glass syringe 200 cc. of blood is transferred from the donor

¹ Howell, W. H., and Holt, E., *Am. J. Physiol.*, 1918, **47**, 328.

² Charles, A. F., and Scott, D. A., *J. Biol. Chem.*, 1933, **102**, 425.

³ Charles, A. F., and Scott, D. A., *J. Biol. Chem.*, 1933, **102**, 431.

⁴ Scott, D. A., and Charles, A. F., *J. Biol. Chem.*, 1933, **102**, 431.

⁵ Murray, D. W. G., Jaques, L. B., Perrett, T. S., and Best, C. H., *Canad. M. A. J.*, 1936, **35**, 621.

⁶ Best, C. H., Cowan, Campbell, and MacLean, D. L., *Science*, 1937, **85**, 338.

⁷ Murray, D. W. G., Jaques, L. B., Perrett, T. S., and Best, C. H., *Surgery*, 1937, **2**, 163.

* Heparin was generously furnished by Dr. C. H. Best, University of Toronto.

† Nembutal was generously furnished by Dr. J. F. Biehn, Abbott Laboratories.

animal into the azotemic one, and then from the azotemic animal to the donor animal. This exchange of blood is carried out 20-40 times. Samples of blood were obtained from each animal before the transfusion, after 20 exchanges of blood and after 40 exchanges. The azotemia of the nephrectomized animal was markedly reduced. Correspondingly, azotemia was produced in the donor animal.

In each of the 6 experiments (only one typical experiment is given in detail) the donor animal's condition was normal the morning after, and the blood urea, which had been elevated, also had returned to a normal level. When the donor animals were sacrificed from one to 3 weeks later their kidneys were found to be normal both grossly and microscopically.

TABLE I.
Experiment 4.

Nephrectomized Dog		Donor Dog (normal)	
Weight 6.8 Kilos.		Weight 10 Kilos.	
Calculated Blood Vol. 566 cc.		Calc. Blood Vol. 833 cc.	
Blood Urea N		Blood Urea N	
in mg. per 100 cc.		in mg. per 100 cc.	
8/18/37, 1 P.M.			
Before bilateral nephrectomy	16.8		
8/19/37, 10 A.M.			
21 hours after nephrectomy	75	Before 1st exchange transfusion	22.5
	61.3	After 1st exchange transfusion	30.5
	54	After 2nd exchange transfusion	36.6
Decrease in blood urea N per 100 cc.	21	Increase in blood urea N per 100 cc.	14.1
Total decrease blood urea N in 566 cc. (Blood Vol.)— 21×5.66	118.8	Total increase in blood urea N in 833 cc. (Blood Vol.)— 14.1×8.33	117.4

Experiments also were carried out using an artificial kidney made of tubes of cellophane immersed in physiological saline similar to the celloidin tubes used by Abel^{8, 9, 10} years ago.

In the experiments so far, from 1 to 4 of these cellophane tubes† were used, each 2 cm. in diameter and 30 cm. in length. Through

⁸ Abel, John J., Rowntree, L. G., and Turner, B. B., *Trans. Assn. Am. Phys.*, 1913, **28**, 51.

⁹ Abel, John J., Rowntree, L. G., and Turner, B. B., *J. Pharm. and Exp. Therap.*, 1914, **5**, 275.

¹⁰ Abel, John J., Rowntree, L. G., and Turner, B. B., *J. Pharm. and Exp. Therap.*, 1914, **5**, 611.

† Cellophane sausage casings were generously furnished by Visking Casing Corp.

a 2-hole rubber stopper tied into one end of the cellophane tube a glass tube was introduced to the bottom of the tube, the bottom end of the cellophane tube having been tied off with twine. Through the second hole in the stopper another glass tube, just long enough to penetrate the stopper leads the blood either back into the animal's vein or through the other cellophane tubes before returning it to the vein. The nephrectomized animal circulated his heparinized blood from his artery through the cellophane tube or tubes back into his vein. In from 3-5 hours 200-700 mg. of urea nitrogen passed from the blood through the cellophane membrane into the surrounding physiological salt solution.

These preliminary experiments suggest the possible use in humans, of either or both of these procedures, since apparently they are harmless. In humans only vein puncture would be necessary, the transfer or circulation of blood to be accomplished by means of a syringe or other mechanical device.

Conditions not infrequently arise in humans as a result of accident, operation or disease which cause previously competent kidneys to become incompetent, leading to azotemia and death. A number of situations of this sort suggest themselves: reflex anuria after an operation on one kidney, post-operative renal insufficiency, anuria in an old prostatic after insertion of an indwelling catheter or after a suprapubic cystotomy preparatory to prostatectomy, anuria in a child suffering a severe, accidental burn, etc.

However, this human application should not be made until further investigation, which is now under way in collaboration with Professor C. H. Best.*

* Since this report was written the work of others has been brought to my attention. Necheles^{11, 12} modified the artificial kidney of Abel successfully, and Haas¹³ used a modified artificial kidney, along with hirudin, successfully to combat temporarily uremia in humans. Nyiri¹⁴ carried out crossed experimental transfusions with paraffin cannulae and without the use of anticoagulants. Azotemia was produced either by bilateral nephrectomy or the injection of uranium nitrate. In some of his experiments thrombi occurred, but several were fairly successful.

¹¹ Necheles, H., *Klin. Wchnschr.*, 1923, **2**, 1257, 1888.

¹² Lim, R. K. S., and Necheles, H., *PROC. SOC. EXP. BIOL. AND MED.*, 1926.

¹³ Haas, Georg, *Klin. Wchnschr.*, 1925, **4**, 13; *Abderhalden's Handbuch der Biol. Arbeit.*, 1929, **5**, 717.

¹⁴ Nyiri, Wilhelm, *Arch. f. Exp. Path. und Phar.*, 1926, **116**, 117.

Cutaneous Reactions to Johnin and Tuberculin in Patients with Cicatrizing Enteritis.

McCHORD WILLIAMS. (Introduced by Elliott C. Cutler.)

From the Surgical Clinic of the Peter Bent Brigham Hospital, Boston, Mass.

The possibility of a relationship between Johne's disease of cattle and human cicatrizing enteritis has been suggested.¹ Johne's disease affects domestic animals, principally cattle, and is characterized by granulomatous lesions of the intestines, lymph nodes, and occasionally the liver.² These lesions resemble those of cicatrizing enteritis³ or the regional ileitis described by Crohn and many others,⁴ except for the constant presence of numerous acid-fast bacilli chiefly in the lesions. These bacilli are generally accepted as the cause of Johne's disease.⁵ From bacilli grown on a synthetic medium which contains no bacterial products, a sterile filtered extract known as Johnin is prepared.⁶ Animals infected with Johne's bacilli react with a rise in temperature to subcutaneous injections of Johnin or avian tuberculin, but not to human tuberculin.⁷ Animals infected with human tubercle bacilli may or may not react to Johnin.⁸

To detect a possible relationship between Johne's disease and cicatrizing enteritis, intracutaneous injections (0.1 cc.) of Johnin, old tuberculin, and 4 control substances were given on the volar surface of the forearm to 5 patients with cicatrizing enteritis diagnosed histologically after operation, and to 5 normal adults. The Johnin was prepared by the U. S. Bureau of Animal Industry. The medium and method of preparation were those used in the manufacture of tuberculin. The concentration was half that of old tuber-

¹ Cutler, E. C., personal communication.

² Hallman, E. T., and Witter, J. F., *J. A. V. M. A.*, 1933, **83**, 159; Johne and Frothingham, *Deutsche Z. f. Tiermed.*, 1895, **21**, 438; Matthews, F. P., *J. A. V. M. A.*, 1930, **76**, 248; M'Fadyean, Sir John, *J. Comp. Path. and Therap.*, 1907, **20**, 48.

³ Dalziel, T. K., *Brit. Med. J.*, 1913, **2**, 1068.

⁴ Crohn, B. B., *et al.*, *J. A. M. A.*, 1932, **99**, 1323; Homans, John, and Hass, George M., *New England J. Med.*, 1933, **209**, 1315; Brown, P. W., *et al.*, *Proc. Staff Meet., Mayo Clin.*, 1934, **9**, 331; Ravdin, I. S., and Rhoades, J. E., *Ann. Surg.*, 1937, **106**, 394.

⁵ Stableforth, A. W., *A System of Bacteriology*, 1930, **5**, 333, Medical Research Council, London.

⁶ Dunkin, G. W., *J. Comp. Path. and Therap.*, 1933, **46**, 59.

⁷ Hagan, W. A., and Zeissig, W., *J. A. V. M. A.*, 1929, **74**, 985.

⁸ Turner, H. W., *J. A. V. M. A.*, 1928, **72**, 1036.

culin.⁹ Reactions to horse serum 1-100, bovine serum 1-100, Frei antigen, and 0.85% saline were negative in every subject except No. 9 (horse serum, 96, and bovine serum, 6). Observations were made at the end of 20 minutes, 8, 24, and 48 hours. Maximal reactions had occurred within 48 hours, and their tabulated size was obtained by multiplying the length by the breadth in cm. of the zone of definite erythema.

TABLE I.

	Patients					Normal Controls				
	1	2	3	4	5	6	7	8	9	
Johnin (undiluted)	82.5	15.0	12.6	24.0	0	22.75	0	.25	0	32.0
Old tuberculin 1-1000	42.5	3.6	2.25	1.04	0	1.5	0	.06	0	.25

These results reveal no relation between non-specific regional enteritis and cutaneous sensitivity to Johnin. Whether the sensitivity to tuberculin associated with sensitivity to Johnin is due to similar components of Johne's and tubercle bacilli or to a non-specific reactivity of tuberculous individuals as described by Hart¹⁰ is a question which awaits further study.

We are indebted to Dr. H. W. Schoening of the Bureau of Animal Industry of the U. S. Department of Agriculture for valuable advice and for a generous supply of Johnin.

9675 P

Gonadotropic Extracts from the Leaves of Young Oat Plants.

MAURICE H. FRIEDMAN.

*From the Department of Physiology, University of Pennsylvania Medical School.**

By the use of the Katzman-Doisy benzoic acid procedure it was found possible to obtain gonadotropic extracts from alfalfa leaf meal.¹ It was, however, difficult to obtain active extracts uniformly from commercial samples available, and it was not found possible to arrange for a dependable supply of alfalfa meal of known age and history. The work of Schnabel² on the biologic value of im-

⁹ Giltner, L. T., U. S. Bureau of Animal Industry, personal communication.

¹⁰ Hart, D., *Quart. J. Med. N. S.*, 1932, **1**, 49.

* This work was supported by a grant from the Rockefeller Foundation.

¹ Friedman and Friedman, *Proc. Soc. Exp. Biol. and Med.*, 1934, **31**, 842.

² Schnabel, C. F., read before the section on biology of the Am. Chem. Soc., New York, April 22, 1935.

mature oat grass prompted an investigation of this material. The young oat leaves proved to be a better source of gonadotropin than alfalfa leaf, and more easy to obtain at the desired stage of growth. The plants were cut when 4 to 6 inches high and either cured in the sun or quickly dehydrated by the method commonly used for drying alfalfa commercially. Two crops were obtained from Kansas and 2 from the New Jersey State Agricultural Experiment Station at New Brunswick.† The extraction procedure has been modified to the extent of substituting 30% acetone for water as the medium for the extraction of the crude protein resulting from the benzoic acid precipitation.

Potent extracts have been repeatedly prepared from each of the 4 successive crops over a period of 3 years. With the present technique the dried oat leaves yield products which produce ovulation in the rabbit by the single intravenous injection of the equivalent of 30 to 80 gm. of the original dry leaf. The latent period, like that of post-coital ovulation, is less than 18 hours and thus differs from the process provoked by the injection of copper salts.³ It is entirely possible that the activity in alfalfa and oat leaves is similar to the thermolabile component in yeast extracts which was found to be active in the hypophysectomized male rat by Hisaw and coworkers.⁴

9676 P

Hyperthyroidism and Vitamin C.*

BARNETT SURE AND ROBERT M. THEIS.

From the Department of Agricultural Chemistry, University of Arkansas.

Recently this laboratory has demonstrated that crystalline vitamin B₁, when administered in large doses will prevent the rapid tissue catabolism and even permit considerable growth in hyperthyroidism in the rat, provided the diet was fortified with an abundance of the stable factor of the vitamin B complex.¹

† The Kansas product was supplied by Dr. Schnabel; the New Jersey samples were supplied through the courtesy and cooperation of Dr. Walter Russell without whose aid these experiments would have been impossible.

³ Fevold, Hisaw, and Greep, *Am. J. Physiol.*, 1936, **117**, 68.

⁴ Hisaw, Greep, and Fevold, *Anat. Rec.*, 1936, **67**, supp. p. 50.

* Research paper No. 476, Journal Series, University of Arkansas, Fayetteville.

¹ Sure, B., and Buchanan, K. S., *J. Nutr.*, 1937, **13**, 513.

During the past 18 months we have been investigating oxidation-reduction efficiency in avitaminosis, which included studies of ascorbic acid concentration in blood, tissues, and endocrine glands. Although it is claimed that the rat either synthesizes vitamin C or requires such minimum amounts that are undetectable by available micro-methods, we are finding appreciable reduction of this vitamin in certain tissues in vitamin B₁ deficiency, thus establishing a definite vitamin interrelationship.

In continuing with the problem of the antithyrogenic action of vitamin B₁ and the possible supplementary value of riboflavin to a ration supplying the stable components of the vitamin B complex in the form of autoclaved beef, it was thought of interest to determine the influence of hyperthyroidism on the ascorbic acid or vitamin C† content of various tissues and endocrines. The experiments were carried out in two series: (1) Five sets in groups of 3 and (2) four sets in groups of 4. The animals were 34 to 53 days of age, ranging in weight from 65 to 101 gm. at the beginning of the experiments. The daily dose of l-thyroxine (Squibbs) was 0.1 mg. and the number of days on thyroxine varied from 10 to 16 days.

The technique employed was that described in a recent communication.¹ As a source of the B vitamins, we used in 4 series of experiments, 500 mg. Northwestern yeast; in three, 40γ to 60γ crystalline vitamin B₁ (Betaxin); and in two, 100γ vitamin B₁ plus 40γ riboflavin. We found that crystalline vitamin B₁ may approximate the protective action of dried Northwestern yeast in hyperthyroidism, from the standpoint of permitting increases in weight in presence of toxic doses of thyroxine, provided enough riboflavin is furnished daily as a supplement to our Ration 2345.¹

When it appeared that the animals receiving thyroxine without vitamin B₁ were near collapse, the entire groups were sacrificed, and vitamin C determinations were made of the adrenals, thymus, thyroids, pituitary, pancreas, liver, kidney, and cerebrum in 3 groups, and heart in 5. For the endocrines we used the micro technique of Farmer and Abt² which they perfected for the blood. Marked and one hundred percent consistent reductions in ascorbic acid content were found in the adrenals, thymus, liver, and kidney. That these marked reductions in ascorbic acid content are not due to heavy losses of body weight became apparent from vitamin B₆ studies, since in the latter experiments we encountered similar losses in weight with no demonstrable change in ascorbic acid in any of

† The terms ascorbic acid and vitamin C are used synonymously in this paper.

² Farmer, C. J., and Abt, A. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 146.

the tissues or endocrines. The reduction in vitamin C in the cerebrum was too small to be significant. There were no changes in the heart, and the results on the thyroid and pituitary are too irregular to warrant any conclusions at this time. In the adrenals there was a reduction of 42 to 44%; in the thymus, 54 to 58% reduction; in the liver 30 to 50% reduction; and in the kidney 47 to 52% reduction in ascorbic acid. These calculations were made from results obtained on fresh tissue.

When there was gain in body weight in presence of thyroxine because of the additions of the B vitamins, there was only 10% reduction in vitamin C content of the adrenals. In the case of the thymus, liver, and kidney, however, the protective action of the B vitamins against vitamin C losses was only partial. Possibly the entire protection against vitamin C losses in the latter endocrine and tissues can be achieved only by vitamin C additions to the rat's diet. Such work is in progress.

It is of interest to note that Aszodi of Hungary has recently found changes in hemoglobin, red blood cells, and differential count in scurvy in guinea pigs to be identical with that of hyperthyroidism produced in the same animal.³ Harris and Ray⁴ reported extreme losses of ascorbic acid in the adrenals of scorbutic guinea pigs. Our similar findings indicate that in experimental hyperthyroidism produced by feeding toxic doses of thyroxine we have produced a vitamin C disturbance in the rat encountered by other investigators in scurvy in the guinea pig. Detailed results will appear *in extenso* elsewhere.

9677 P

Protective Effects Against Lethal *Streptococcus hemolyticus* of Glutamine and Various Sulfonamide Compounds.

CHARLES WILLIAM SCHAFFER. (Introduced by R. Beutner.)

From the Department of Pharmacology, The Hahnemann Medical College, Philadelphia.

A total of 72 rabbits were inoculated with 1.0 cc. of a suspension of *Streptococcus hemolyticus*, sufficiently virulent to produce lethal effects within one week or less. Animals weighing approximately

³ Aszodi, Z., *Klin. Wochenschrift*, 1937, **20**, 715.

⁴ Harris, L. J., and Ray, S. N., *Biochem. J.*, 1933, **27**, 303.

2 kg. each were used; all had the same diet; droppings were screened, and cages, screens, and pans were sterilized daily. The animals were divided into 6 groups of 12 each, and 48 hours after inoculation, the first 5 groups were given initial intravenous injections with 1.0% solutions of para-amino-glutamine, D-glutamine, phenyl-alanine sulfonamide, sulfanilamide (prontylin), and para-tolyl-sulfonamide, respectively, *i. e.*, one drug to each group. The sixth group was used as a control. In the first 5 groups 2.0 cc. doses, given intravenously, were administered 4 times daily for 6 days, and the deaths and survivals were recorded for each group.

TABLE I.

Group*	Drug (administered 48 hrs. after inoculation)	Chemical Formula	Effect of daily dosage 1% solution in 8 cc. per animal for 6 days		
			No. of animals given drug	Deaths	Survivals after one month
1.	D-glutamine	$ \begin{array}{ccccccc} & \text{H} & \text{H} & \text{H} & & \text{O} & \\ & & & & & & \\ \text{COOH} & -\text{C} & -\text{C} & -\text{C} & - & \text{C} & \\ & & & & & & \\ & \text{NH}_2 & \text{H} & \text{H} & & \text{NH}_2 & \end{array} $	12	12	0
2.	Para-amino-glutamine	$ \begin{array}{ccccccc} & \text{O} & \text{H} & \text{H} & \text{H} & & \text{O} \\ & & & & & & \\ \text{NH}_2 & -\text{C} & -\text{C} & -\text{C} & -\text{C} & - & \text{C} & -\text{NH}_2 \\ & & & & & & & \\ & \text{NH}_2 & \text{H} & \text{H} & \text{H} & & \text{NH}_2 & \end{array} $	12	12	0
3.	Phenyl-alanine sulfonamide	$ \begin{array}{c} \text{CH}_2\text{CH} \cdot \text{NH}_2\text{C} \begin{array}{l} \text{O} \\ \\ \text{OH} \end{array} \\ \\ \text{C}_6\text{H}_5 \\ \\ \text{SO}_2\text{NH}_2 \end{array} $	12	0	12
4.	Sulfanilamide (Prontylin)	$ \begin{array}{c} \text{NH}_2 \\ \\ \text{C}_6\text{H}_4 \\ \\ \text{SO}_2\text{NH}_2 \end{array} $	12	3	9
5.	Para-tolyl-sulfonamide	$ \begin{array}{c} \text{CH}_3 \\ \\ \text{C}_6\text{H}_4 \\ \\ \text{SO}_2\text{NH}_2 \end{array} $	12	6	6
6.	None—Control		0	12	0

* There were 12 animals in each group, and all 72 in all 6 groups were inoculated with ordinarily lethal doses of *Streptococcus hemolyticus*.

Tabulation of the relative protective action of these drugs against ordinarily lethal doses of *Streptococcus hemolyticus* shows the following (Table I).

The Prontylin and para-tolyl-sulfonamide were selected because of the former's (NH_2) and the latter's (CH_3) groups. The results in these experiments show that in these drugs the substitution of the (CH_3) for (NH_2) in sulfanilamide reduced, to a definite extent, the protective action of this drug. Phenyl-alanine-sulfonamide was found to protect rabbits from ordinarily lethal inoculations of *Streptococcus hemolyticus* even more completely than sulfanilamide; this substance did not show any toxicity in the doses given while sulfanilamide definitely showed some toxicity. The acid amide group ($\text{CO} \cdot \text{NH}_2$) has no protective action compared with the glutamine structures.

Conclusions. 1. The protective action induced in rabbits against the lethal effects of *Streptococcus hemolyticus* infections depends chiefly on the presence of the SO_2NH_2 group, not so much on the free NH_2 group. 2. Phenyl-alanine-sulfonamide was found to exert a more complete protective action against ordinarily lethal inoculation in rabbits of *Streptococcus hemolyticus* suspensions than the better known sulfanilamide.

9678 P

Absorption of Insulin by Yeasts.

L. ROSENTHAL AND J. KAMLET.

From the Department of Laboratories, Israel Zion Hospital, Brooklyn, N. Y.

The absorption experiments were performed in the following manner: a cake of fresh yeast (Fleischmann) was suspended in 40 cc. of physiological salt solution, vigorously shaken for a few minutes, and centrifuged. The supernatant fluid was discarded. This procedure was repeated in order to remove extraneous matter present in the cake. The washed yeast cells were tested on a slide for viability by mixing a drop of the yeast suspension with a drop of Loeffler's methylene-blue solution and examining microscopically. Viable cells remained unstained, while dead cells readily took up the dye. Usually, only about 5-10% of the cells were stained. If the percentage of dead cells was higher, the cake was not used.

The washed yeast cells were suspended in a mixture of 20 cc. of

insulin U-40 of pH 3.5 and 60 cc. of saline. The suspension containing 800 units of insulin, or 10 units per cc., was allowed to stand for 2 hours at room temperature, occasionally stirred, and then centrifuged for 5 minutes at 1200 r.p.m. The supernatant fluid was decanted from the sediment and the latter washed once with 80 cc. saline in order to remove traces of the supernatant fluid, and then suspended in sufficient saline to make 80 cc. The insulin content of both the supernatant fluid and yeast cells suspension was determined in the usual way by injection into a series of fasting rabbits. The testing was performed several times with freshly prepared mixtures of insulin and yeasts. One cc. of the sediment suspension induced hypoglycemic shock in 12 of a series of 14 rabbits in from 2 hr. 10 min. to 4 hr. 50 min. The drop in blood sugar varied from 58 to 114 mg. %, with an average fasting glycemia of 98 mg. %, and an average glycemia during the shock of 24 mg. %. The minimal dose of yeast cell suspension which caused shock in 9 out of 12 fasting rabbits was 0.4 cc. to 0.6 cc. Inasmuch as that amount, according to the existing standard, corresponded to 3 units we may assume that 1 cc. of the suspension contained approximately 5-7.5 units of insulin. Control injections, with non-treated yeasts, failed to produce any change in the blood sugar.

The supernatant fluid was likewise bio-assayed for insulin content. 1.5 cc. injected into a series of 20 rabbits caused insulin shock in 15 animals in from 25 min. to 2 hr. 10 min. The drop in blood sugar varied from 64 to 88 mg. %, with an average fasting glycemia during shock of 32 mg. %. The minimal doses of supernatant fluid required to induce insulin shock in 12 out of 20 fasting rabbits was 0.6 cc. to 1.5 cc., thus showing that the supernatant fluid contained from 2 to 5 units per cc. instead of the original 10 units.

By comparing the insulin content of the yeast cells with that of the supernatant fluid we may assume that the yeast cells absorb from 50% to 75% of the insulin of a solution assaying 10 units per cc.

Substantially the same results were obtained when the absorption experiments were performed at 20° and 37° or by overnight incubation in the refrigerator. At 45° no absorption took place. Yeasts killed by heating at 100° or by chloroform did not show any evidence of insulin absorption.

Thus it follows that only viable yeast cells are able to absorb insulin. We are at present investigating the effect of peroral administration of "insulinated" yeast.

Effect of Chloride on the Thyroid Gland.*

ROE E. REMINGTON.

From the Department of Chemistry, Medical College of the State of South Carolina, Charleston.

Hibbard has reported¹ hyperplasia of the thyroid with deficiency of colloid in rats given 2% of calcium chloride or 3% of sodium chloride in the drinking water. The diet consisted of pearled barley, which was low in iodine. Rats given distilled water without the added chloride had histologically normal thyroids. The results were interpreted as indicating that excess of chloride interferes with the utilization of minimal amounts of iodine in maintaining normal thyroid structure.

In the technique devised by the writer and his associates² for the quantitative measurement of the effects of iodine deficiency on the thyroid gland, judgment is based on 3 factors, *viz.*, weight, dry matter and iodine content of the glands. In applying this method to the study of the effect of the chloride ion, the iodine deficient basal diet (diet 342) was varied by changing the salt content from 0 to 4%, and fed to parallel groups of young rats weighing 40-50 gm. for 6 weeks. Each group consisted of 6 animals, equally divided as to sex. The results are shown in Table I.

TABLE I.

Diet No.	Salt, %	Gain in weight, gm.	Food consumed, gm. for each gm. of gain	Thyroid Glands		
				Fresh weight, mg. per 100 gm. body wt.	Dry matter, %	Iodine, dry basis, %
357	0	39.3±1.7	9.72	18.6±0.8	27.0	.049
342	1	90.3±1.9	4.86	18.9±0.4	24.9	.016
358	2	105.0±4.3	4.75	18.9±0.4	24.2	.016
359	4	87.0±3.0	5.25	19.0±0.6	22.7	.013
Colony		125.0	4.01	10.0	32.0	.200

Rats receiving no salt made very poor growth in comparison with the others, notwithstanding their food consumption per gram gain in weight was twice as great. This poor growth and high food con-

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

¹ Hibbard, J. S., *Arch. Surg.*, 1937, **26**, 648.

² Remington, R. E., *J. Nutrition*, 1937, **12**, 223.

sumption probably accounts for the higher dry matter and iodine content of the glands, since the diet is not entirely devoid of iodine. The normal values for rats of similar age in our colony are: thyroid weight 10 mg. per 100 gm. body weight, dry matter 32%, iodine on dry basis 0.200%.³ When compared with these values, the rats of all groups had goiter, and increasing chloride up to the point where it began to retard growth did not affect the severity of the condition, with the possible exception noted above. That the enlargement is not as great as has been reported in previous experiments is probably to be attributed to variations in the iodine content of the yellow corn meal.

9680

Lipid Content of Blood Leucocytes Following Hemorrhage in Rabbits.

ELDON M. BOYD, J. W. STEVENSON AND E. A. WATKINSON.

From the Department of Pharmacology, Queen's University, Kingston, Canada.

Harlow and Selye¹ reported that a neutrophilic leucocytosis occurred in rats and mice following the administration of various acute, non-specific, damaging stimuli and they considered that this leucocytosis was part of a general response to such types of stimuli, a response which they described as an "alarm reaction". The "alarm reaction," according to Selye,² represents the first stage of a "general adaption reaction," the "stage of alarm" being followed by a "stage of resistance" and finally, if sufficient stimulation be continued, by a "stage of exhaustion." During the course of certain experiments,³ one-quarter of the total blood volume was removed from rabbits and it was thought that such a stimulus might provide an "alarming stimulus" and evoke an "alarm reaction." This amount of blood was taken as quickly as possible by either cardiac puncture or from the marginal ear veins and constituted a distinct shock to the animal; it was the largest amount of blood which could be removed thus and not cause a considerable number of deaths; even removing this amount produced an occasional death.

³ Remington, R. E., Remington, J. W., and Welch, S. S., *Anat. Rec.*, 1937, **67**, 367.

¹ Harlow, C. M., and Selye, H., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 141.

² Selye, H., *Endocrinol.*, 1937, **21**, 169.

³ Boyd, E. M., and Stevenson, J. W., *J. Biol. Chem.*, 1937, **122**, 147.

Total and differential leucocyte counts were done on these animals before and after bleeding. The phospholipid and free cholesterol contents of the blood leucocytes were simultaneously estimated by methods previously used.⁴ Thirty young, healthy rabbits were employed, 30-40 cc. of blood removed without anesthesia and a second sample of the same volume taken after an interval of 3 hours in one group of 6 animals, after 6 hours in a second group, and after 12, 24, and 48 hours in 3 further groups.

The hemorrhage produced a consistent decrease which averaged 25% in the concentration of hemoglobin and in the red cell count with no evidence of regeneration of red cells or hemoglobin up to and including the 48-hour interval. The mean values and their standard deviation of the total white cell count, phospholipid and free cholesterol of the blood leucocytes before bleeding are given in Table I. The mean values only are listed at the various inter-

TABLE I.
The Mean Phospholipid and Free Cholesterol Content of the Blood Leucocytes Following Bleeding in Rabbits.
Lipid values are expressed in mg. per 100 gm. moist weight.

Hours after Bleeding	Total Leucocyte Count	Phospholipid	Free Cholesterol
0	8,450±2,030	944±424	281±122
3	8,800	1,243	257
6	8,100	1,444	473
12	8,100	884	308
24	12,000	1,150	261
48	8,800	846	273

vals after bleeding since it is obvious that no statistically significant change could occur with the slight and variable differences in these latter means. The total white cell count did not rise appreciably above nor fall appreciably below the initial, indicating that the leucocytes lost during bleeding were rapidly replaced (in less than 3 hours) but that no leucocytosis occurred up to 48 hours after bleeding. There was likewise no consistent change in the differential count with the exception that within the first 6 hours after bleeding all of the animals showed relatively more polymorphs and the lymphocytes increased in number between the 12th and 24th hours.

There was no consistent change in either the phospholipid or free cholesterol content of the white blood cells following bleeding. By usual statistical methods, the values of free cholesterol at 6 hours were significantly elevated but this did not appear likely in view of

⁴ Boyd, E. M., Blenkinsop, J. W., and Mylks, G., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 300.

the other results. It would thus appear that the leucocytes mobilized into the blood stream following hemorrhage have the same lipid composition as those previously present.

Summary. Removal of one-quarter of the blood volume of rabbits produced no appreciable change during 48 hours in the phospholipid and free cholesterol content of the white blood cells and no leucocytosis.

9681 P

Transfer of Acquired Resistance to Transplantable Leukemia in Mice.*

J. S. POTTER, M. J. TAYLOR AND E. C. MACDOWELL.

From the Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, L. I., N. Y.

It has been demonstrated that graduated doses (cell-immunization) of leukemic cells¹ and certain normal tissues^{2, 3} can induce resistance to transplantable leukemia in normally susceptible mice. Following cell-immunization injected leukemic cells immediately develop small lesions which soon become necrotic.⁴ Lesions do not form immediately following leukemic inoculation of strain C58 mice made resistant by Sto-Li foetal tissues. Once resistance to line I leukemia has been established by means of graduated cell doses it is permanent, while resistance caused by normal tissue implantation may be only temporary with a peak of effectiveness in C58 mice 3 days after implantation of Sto-Li tissue (unpublished data). Of further interest in this problem is the question: Can resistance induced by these 2 methods be transferred to normally susceptible mice?

Accordingly, spleen and liver were removed from cell-immunized mice, minced and injected into susceptible C58 mice. Another

* Aided by a grant from the Carnegie Corporation.

¹ MacDowell, E. C., Taylor, M. J., and Potter, J. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **32**, 84.

² MacDowell, E. C., Taylor, M. J., and Potter, J. S., *Proc. Nat. Acad. Sci.*, 1935, **21**, 8, 507.

³ Rhoads, C. P., and Miller, D. K., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 817.

⁴ Potter, J. S., and Findley, M. D., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1338.

group of C58 mice was given injections of Sto-Li foetal tissues and 3 days later the spleens were removed from these animals, minced and injected into susceptible C58 mice. Three days after receiving transplants from resistant animals both groups of mice were injected with a normally lethal dose of line I transplantable leukemia. C58 mice treated with normal C58 spleen were also given leukemic inoculations to verify the observed lack of resistance to line I leukemia following implantation of C58 tissue in C58 mice.² Control inoculations of normal C58 mice were made to check the potency of the dose of leukemic cells. A summary of the results of these experiments is given in Table I.

TABLE I.
Summary of Experiments on Passage of Resistance to Inoculation with Line I
Transplantable Leukemia in Strain C58 Mice.

0.1 cc. minced transplanted tissue	No. of mice	Died	Survived
Spleen of cell-immunized mice	27	1*	26
Liver of cell-immunized mice	5	0	5
Spleen of Sto-Li tissue-immunized mice	10	10 (5.48)†	0
Spleen of normal C58 Mice control	17	17 (5.15)†	0
Untreated controls	16	16 (5.5)†	0

*Died at 2 days negative for leukemia.

†Numbers in parentheses indicate average interval before death in days.

All animals given dose of leukemic cells calculated $1,000,000 \pm$ from sample counts of the inoculum. Over a period of 3 years 280 normal C58 mice have been given this dilution with no survivors.

These preliminary results demonstrate that resistance to a transplantable leukemia may be transferred from cell-immunized mice to normal mice by implantation of tissue from actively immunized mice. Under the conditions of this experiment resistance induced by normal tissues was not transferable. Quantitative effects, duration of the transferred resistance and use of cell-free material are questions for further experiments.

9682 P

Antigenicity and Infectivity of Extracts of Virus-Induced Rabbit Papillomas.

JOHN G. KIDD.

From the Laboratories of the Rockefeller Institute for Medical Research.

The antigen which binds complement when mixed with sera neutralizing the Shope papilloma-virus bears an evident relation to the virus itself; for it is present in high titer in virus-containing extracts of papillomas, it is retained by filters which hold back the virus, and its effectiveness is destroyed by the same amount of heating which renders the virus non-pathogenic.¹ The experiments now to be reported throw further light on this relation.

Tests with Extracts of Non-Infectious Papillomas and of Normal Skin. On inoculation the Shope virus gives rise to papillomas in both domestic and cottontail rabbits; yet only infrequently can it be extracted in active state from the domestic-rabbit growths, and occasionally those of cottontails fail to yield it. Papillomas from 6 cottontail and 8 domestic rabbits, which were produced by the virus yet from which it could not be recovered, have been extracted as usual and tested repeatedly for capacity to bind complement under optimal conditions. Most of these non-infectious extracts failed to bind complement, but a few bound it in a very low titer. Extracts of the normal skin of 3 cottontail rabbits also failed to bind complement, whereas the highly-infectious extracts of their naturally occurring papillomas bound it completely on concurrent test.

Antigenicity of Extracts of Non-Infectious Papillomas. Shope has found that extracts of virus-induced papillomas, which yield no active virus on extraction, will elicit virus-neutralizing antibodies when injected intraperitoneally into rabbits of homologous species.² He has generously sent sera from 9 domestic rabbits immunized in this way for complement-fixation tests. They bind complement when tested with suitable antigens, and their capacity to do so varies directly with their ability to neutralize the virus. The sera of 3 normal domestic rabbits failed to bind complement in the same test, as have all other normal sera tested under like conditions.

In order to elicit antibodies with extracts of non-infectious papillomas, crude suspensions of their tissue must be used, Berkefeld V filtrates of these suspensions having proved devoid of antigenicity, in my experience, even when large amounts were injected.

¹ Kidd, John G., *Proc. Soc. Exp. Biol. and Med.*, 1937, **35**, 612.

² Shope, R. E., *J. Exp. Med.*, 1937, **65**, 219.

Effect of Irradiation with Ultraviolet Light. The papilloma-virus is very resistant to ultraviolet light, perhaps because of adaptation consequent on its exposure to sunlight under natural conditions. Exposure of the fibroma-virus for less than 5 minutes under a mercury-vapor lamp* at a distance of 48 cm. renders it completely non-infectious;³ while irradiation for 20 to 90 minutes under the same conditions has been found necessary to reduce or abolish the infectivity of the papilloma-virus in potent 10% extracts. Such exposures did not diminish the complement-binding capacity of these materials, however, nor did irradiation for 120 minutes.

Effect of Variations in pH. The infectivity of papilloma-extracts is known to be immediately destroyed at certain pH levels, slowly at others.⁴ To learn the reaction of the complement-binding antigen to variations in pH, portions of two extracts were incubated at 37°C. for 14 hours at various pH levels, with adjustment of all to pH 6.8 thereafter. At pH 4.4 and 9.0, both materials remained highly infectious and capable of binding complement completely, as also at 6.8; whereas at pH 1.8, 2.8, and 10.5 both the pathogenicity and complement-binding capacity of the extracts were abolished. At pH 9.8, however, the extracts lost almost completely their infectious properties, but retained undiminished their capacity to bind complement.

The fixation of complement under the conditions described manifestly involves a specific antigen-antibody union—a reaction, that is to say, which is specific in the rigid immunological sense. The complement-binding antibodies are elicited under the same conditions as the virus-neutralizing, and in the same relative proportions; while the complement-binding antigen is very closely related to the virus, perhaps identical with it. Drastic procedures (heating, treatment with strong acid or strong alkali) abolish both the infectivity and complement-binding capacity of papilloma extracts; but less injurious measures (irradiation with ultraviolet light, treatment with weak alkali) can render extracts non-pathogenic without destroying their complement-binding capacity.

* Alpine Sun Lamp, Hanovia Chemical Co.

³ Rous, P., McMaster, P. D., and Hudack, S. D., *J. Exp. Med.*, 1935, **61**, 657.

⁴ Wyckoff, R. W. G., and Beard, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1937, **36**, 562.

Chemical Studies of the Intrinsic Factor in Desiccated Stomach and Normal Human Gastric Juice. I. Separation of the Intrinsic Factor.

S. M. GOLDHAMER AND JEAN KYER. (Introduced by R. Isaacs.)

*From the Thomas Henry Simpson Memorial Institute for Medical Research,
University of Michigan, Ann Arbor.*

In previous reports it was demonstrated that the intrinsic factor of Castle was present in the gastric juice of individuals with pernicious anemia¹ as well as in the gastric contents of normal individuals. It was also shown that desiccated stomach contained both the extrinsic and intrinsic factors.² The object of this paper is to report some results of the fractionation of desiccated stomach and of normal gastric juice in an attempt to isolate the intrinsic factor and determine its nature.

All the subjects used in the experiments were proven to have true pernicious anemia by the history, physical findings and laboratory procedures. To each of these patients either various fractionation products of desiccated stomach or gastric juice incubated with beef steak were fed daily at 8 A. M. No food was permitted for at least 8 hours before this time or for 5 hours afterwards. Blood observations were made daily with U. S. Bureau of Standards equipment. Reticulocyte counts were made from brilliant cresyl blue film preparations; one thousand red cells were counted.

An acidified water extract (pH 4.5) of desiccated hog stomach was prepared² and, after concentration by forced air drying at room temperature, the remaining clear yellow fluid was saturated with ammonium sulfate. The precipitate was removed by filtration and dialyzed in a Parlodion sac against distilled water for several days to remove the ammonium sulfate. The contents of the sac were then removed, dried, and fed daily to a patient with pernicious anemia in relapse (red blood cells 1,700,000 per cu. mm.; hemoglobin 64%). The daily dose was that amount of dried precipitate equivalent to 50 gm. of hog stomach. After 7 days of therapy a slight reticulocyte response was noted, indicative of a subminimal potency.

The same patient was then fed the above described precipitate to-

¹ Goldhamer, S. M., *Am. J. M. Sc.*, 1936, **191**, 405.

² Kyer, J., Brooks, F. P., and Isaacs, R., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 677.

gether with the boiled acid extract of desiccated stomach which had been shown to contain only the extrinsic factor.² On the eighth day of treatment the reticulocytes reached a peak of 13.7% and the red blood cells on the tenth day increased to 2,500,000 per cu. mm.

The above experiments demonstrated the possibility of the separation of the intrinsic and extrinsic factors of hog stomach by the use of ammonium sulfate and dialysis, and further suggested a method of salting out the intrinsic factor from human gastric juice. The following experiments were performed for this purpose. Our primary intent was to further the isolation of the intrinsic factor and not to prepare a material of any specific degree of potency.

Normal filtered gastric juice was brought to pH of 4.5 with concentrated sodium hydroxide and then saturated with ammonium sulfate. The precipitate was removed, dialyzed for 8 hours, and dried at room temperature. A half gram of the dry powder representing 200 cc. of normal gastric juice was mixed with 200 cc. of water acidified with hydrochloric acid to a pH of 2.0, incubated for 3 hours at 37°C., and fed daily for 7 days to a patient with pernicious anemia in relapse (red blood cells 2,270,000 per cu. mm.; hemoglobin 63%). The subject was permitted no food for at least 8 hours before treatment or 5 hours afterwards. No response occurred during a 12-day period.

This same patient was then fed the identical preparation as described in the previous experiment with the addition of 200 gm. of ground beef steak to supply the extrinsic factor. The mixture was incubated for 3 hours at 37°C. with 200 cc. of water acidified with hydrochloric acid to a pH of 2.0 before administration to the patient. The treatment was continued for 7 days. Although the reticulocytes did not reach the calculated maximum response, a peak of 8.0% was observed on the fifth day after therapy was instituted.

The preceding experiment was repeated to rule out the possibility of a spontaneous remission. A patient with pernicious anemia in relapse (red blood cells 1,740,000 per cu. mm.; hemoglobin 35%) was fed daily a mixture of 0.78 gm. of dry powder (200 cc. normal gastric juice) incubated with 200 gm. of ground beef steak and 200 cc. of acidified water at 37°C. for 3 hours. This treatment was continued for 9 days. A reticulocyte peak of 24% was observed on the sixth day. This experiment adds supportive evidence to the previous one and demonstrates the possibility of removing the intrinsic factor from normal gastric juice by precipitation with ammonium sulfate and dialysis.

*Conclusions.** 1. The intrinsic factor which is present in desiccated hog stomach and normal gastric juice may be salted out from a solution saturated with ammonium sulfate. 2. The intrinsic factor after salting out with ammonium sulfate is not dialyzable.

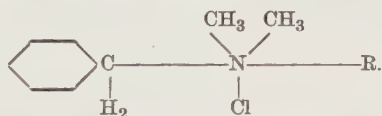
9684

Fungicidal Properties of Alkyl-Dimethyl-Benzyl Ammonium Chlorides.*

CECIL G. DUNN. (Introduced by J. W. Williams.)

From the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge.

The organic compound, alkyl-dimethyl-benzyl ammonium chlorides, is a new type of germicide having the following formula:



R represents a mixture of the alkyl radicals C_8H_{17} , $\text{C}_{10}\text{H}_{21}$, $\text{C}_{12}\text{H}_{25}$, $\text{C}_{14}\text{H}_{29}$, $\text{C}_{16}\text{H}_{33}$, and $\text{C}_{18}\text{H}_{37}$ as derived from the fatty acids of coconut oil.

Some of the germicidal, antiseptic, physical and chemical properties of this rather unusual germicide have been reported by Domagk,¹ Heineman,² and Dunn.^{3, 4}

The test organisms used in the present investigation were secured from the following sources: *Actinomyces gedanensis* and *Cryptococcus hominis* from the American Type Culture Collection, and the others from stock collections maintained by the Department of Biology and Public Health.

The fungi were grown in Sabouraud's dextrose media and subcultured in Sabouraud's dextrose broth, with the exception of *Micro-*

* The conclusions obtained are in agreement with those of Helmer and Fouts³ who published a similar report during the preparation of this paper.

³ Helmer, O. M., and Fouts, P. J., *Am. J. M. Sc.*, 1937, **194**, 399.

*Contribution No. 112 from the Department of Biology and Public Health. Research aided by a grant from the Alba Pharmaceutical Company, Inc.

¹ Domagk, G., *Deut. med. Wochenschr.*, 1935, **21**, 829.

² Heineman, P. G., *J. Am. Pharm. Assn.*, 1937, **26**, 711.

³ Dunn, C. G., *Proc. Soc. Exp. Biol. and Med.*, 1936, **35**, 427.

⁴ Dunn, C. G., *Am. J. Hyg.*, 1937, **26**, 46.

sporon audouini, which was grown and subcultured in Sabouraud's maltose broth principally, and *Act. gedanensis*, which was grown and subcultured in standard Reddish broth. The fungi were incubated at 30°C., with the exception of *Monilia albicans*, which was grown at 37°C.

Suspensions of the molds were prepared from the broth cultures by transferring the contents of one or more tubes† to a sterile glass bottle containing sterile glass beads, or broken glass, and shaking the bottle vigorously by hand for 5 minutes. A few suspensions were prepared from cultures grown on solid media, as described by the author previously.⁵ The suspension thus obtained was placed in a medication tube and cooled or heated to the temperature of the test. It was used as prescribed by F. D. A. Methods,⁶ generally after standing for 15 minutes, or less.

A 1/1,000 tincture of alkyl-dimethyl-benzyl ammonium chlorides was employed in the fungicidal tests. This tincture contained 50 cc. of ethyl alcohol (95%), 10 cc. of reagent acetone, the ammonium compound (0.1 gm.), and distilled water, q.s. 100 cc.

Results of fungicidal tests are given in Table I. Each value rep-

TABLE I.
Action of a 1/1000 Tincture* of Alkyl-Dimethyl-Benzyl Ammonium Chlorides
Against Some Fungi

Test organisms	Average phenol coefficients		Aver. highest dilution destroying test organism in 10 but not 5 min.§			
			Ammonium		Ammonium	
	20°C.	37°C.	Phenol compound 20°C.	Phenol compound 37°C.	Phenol compound 20°C.	Phenol compound 37°C.
<i>Achorion schoenleinii</i>	.2—	.36†	1/87	1/17	1/140	1/50
<i>Actinomyces gedanensis</i>	1.0	1.3	1/130	1/130	1/205	1/275
<i>Cryptococcus hominis</i> ‡	.21	.4	—	1/24	—	1/70
<i>Epidermophyton inguinale</i>	.36	.44	1/123	1/45	1/167	1/73
<i>Microsporon audouini</i>	.074	.12±	1/175	1/13	1/320±	1/35
<i>Microsporon lanosum</i>	.089†	—	1/90	1/8	—	—
<i>Monilia albicans</i>	.17	.60	1/77	1/13	1/93	1/55
<i>Saccharomyces cerevisiæ</i>	.09	.15	1/100	1/9	1/135	1/21
<i>Trichophyton interdigitale</i> , No. 1	.1—	.28†	1/60	1/6	1/90	1/25
<i>Trichophyton interdigitale</i> , No. 2	.12	.28†	1/63	1/7	1/90	1/25

* Alcohol-acetone—aqueous solution.

† One test only.

‡ Figures based on 1/1000 aqueous solution of the ammonium compound.

§ Dilutions of the 1/1000 solution.

† Bacterial culture tubes.

⁵ Dunn, C. G., *J. Inf. Dis.*, 1937, **61**, 31.

⁶ Food and Drug Administration Methods of Testing Disinfectants and Antiseptics, Circular No. 198, U. S. D. A., 1931.

resents the average of from 2 to 5 individual tests, unless otherwise specified.

An examination of the table shows that *Act. gedanensis*, *E. inguinale*, *C. hominis*, and *Ach. schoenleinii* were most readily destroyed by the ammonium compound at both 20°C. and 37°C. The two strains of *T. interdigitale* were the most resistant of the pathogenic molds both to the ammonium compound and to phenol. Dilutions of the 1/1,000 tincture of the ammonium compound were effective in destroying all of the pathogenic fungi examined under the conditions of the tests.

In the presence of 20% horse serum, *E. inguinale*, *Ach. schoenleinii*, and *Act. gedanensis* were readily destroyed by dilutions of the 1/1,000 tincture of the ammonium compound.

Cup-plate tests were carried out as suggested by F. D. A. Methods, with the exception that 0.2 cc. of germicide was added to each cup instead of the 6 drops advocated. This modification was adopted in order to insure greater accuracy and uniformity of results.

The 1/1,000 tincture of alkyl-dimethyl-benzyl ammonium chlorides was used in the tests. Fairly good results were obtained against *Ach. schoenleinii* and *C. hominis*, but the action against *Monilia albicans*, though effective, was of low order.

Other solvents for the ammonium compound were used in an effort to improve its penetration in agar. The use of 50% tertiary butanol as the solvent for the ammonium compound increased the areas of the clear zones in every case. When alkyl-dimethyl-benzyl ammonium chlorides were dissolved in full-strength tertiary butanol to form a 1/1,000 dilution of the ammonium compound, the results obtained with *C. hominis* and *Monilia albicans* were superior to those secured by using the 50% tertiary butanol. Thus tertiary butanol was shown to be a better solvent for alkyl-dimethyl-benzyl ammonium chlorides than the alcohol-acetone solution where cup-plate tests were concerned.

Studies on *Aedes aegypti* Infected in the Larval Stage with the
Virus of Yellow Fever.*

LORING WHITMAN AND P. C. A. ANTUNES. (Introduced by J. H.
Bauer.)

From the Laboratory of the Yellow Fever Service at Bahia, Brazil.

During the course of general transmission studies with *Aedes aegypti*, it became of interest to see whether these mosquitoes could be infected in the larval stage, and retain the infection into adult life. To this end, half-grown larvæ were placed in a bowl containing a mixture of blood serum from a Rhesus monkey at the height of infection with the Asibi strain of yellow-fever virus, and an equal quantity of physiological salt solution. This was shown, by titration in mice, to contain 30,000,000 minimum lethal doses for mice per cc. Pupation commenced after 48 hours. The pupæ were washed and suspended individually in tubes of clean water, and the adults began to emerge 4 days after the first contact with the virus. The first 28 males to emerge were ground up and injected into a Rhesus monkey subcutaneously on the day of emergence. The monkey responded 4 days later with fever, and had virus in its blood stream. It was killed when moribund on the sixth day. Necropsy findings, both macroscopic and microscopic, were typical of yellow fever. Four days later, 15 females, varying in age from one to 4 days, were fed on a normal Rhesus, but failed to infect it. However, 10 days later 29 females from the same lot were able to produce yellow fever by bite in another Rhesus monkey.

To confirm this observation, half-grown larvæ were again placed in 50% serum from an infected Rhesus. This mixture was shown to contain 7,600,000 M.L.D. for mice per cc. In order to prevent the mosquitoes from pupating in the viral suspension, the larvæ were washed 3 times in water at the end of 24 hours, and placed in a bowl of clean water. Pupation commenced the following day, and the first adults appeared 2 days later. Thirty of the first males to emerge were ground up on the day of emergence and the suspension was titrated in mice, by the technic described by one of us.¹

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the Cooperative Yellow Fever Service, maintained by the Brazilian Ministry of Education and Health and the International Health Division of The Rockefeller Foundation.

¹ Whitman, L., *J. Exp. Med.*, 1937, **66**, 133.

Virus was present though the titer was low (284). Thirty more males were tested at 7 and another 30 at 14 days after emergence. The titers had increased considerably (72,500 and 127,500, respectively). In the meantime, 25 four-day-old females fed on a Rhesus monkey, which died of yellow fever.

Using the same technic, we have infected mosquitoes with the mouse-brain-adapted neurotropic strain of the French virus in its 530th to 540th passage in mice.

At this time, it was of interest to determine whether larvæ could be infected when new born and retain the virus throughout the entire aquatic stage. To study this, eggs were placed in serum virus mixtures, and after a few hours those which had not hatched were removed. All resultant larvæ thus had approximately the same exposure to the virus. On the following day, the larvæ were washed 3 times before being placed in a bowl of clean water. Unfortunately, in 3 such experiments the titer of virus in which the eggs were hatched was low, and the adults were subsequently shown to be non-infectious. However, one lot, infected in a suspension of neurotropic virus containing 33,000,000 M.L.D. for mice per cc., was shown, by titration in mice, to have retained the virus during the 7 to 8 days between washing and emergence. From this it would seem that unusually large initial infections are necessary for the larvæ to retain the virus throughout the entire aquatic stage.

The possibility of the transmission of virus through the egg was also considered in this series of experiments. It was thought that, since by the above method males as well as females could be infected, virus might be introduced into the embryos either through the spermatozoa or through the ova, or both. To test this theory, newly-emerged infected males and females were mated, and the females allowed to feed on normal guinea pigs to promote oviposition. Ten days later the first lot of eggs was removed. However, on the assumption that time was necessary for testes and ovaries to become infected, a second lot of eggs was collected over the next 15 days. These had been produced between the tenth and twenty-fifth days of infection. Both batches of eggs were placed in water, and eventually the resultant adults were ground up and injected into both mice and monkeys. No virus was recovered from them.

From this it can be seen that *Aedes aegypti* can be infected in the larval stage with the virus of yellow fever, providing they are exposed to large quantities of virus. The resultant adults, both male and female, are infected. Whether the infection produced in this way is identical with an infection following the ingestion of virus by

feeding during adult life remains to be seen. Nevertheless, this method of infecting mosquitoes offers possibilities for the study of transmission, particularly with insects which can be brought to the laboratory in the egg or larval stage.

9686

Vascular, Metabolic, and Motility Responses of Uterine Tissue Following Administration of Oestrin.

JOHN MACLEOD AND SAMUEL R. M. REYNOLDS.*

From the Biological Laboratory, Cold Spring Harbor, Long Island, and the Department of Anatomy, University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.

Within 30 minutes after the administration of oestrin to ovariectomized rabbits, a maximal hyperemia of the uterine vascular bed takes place.¹⁻⁴ Rhythmic motility of the myometrium does not commence until about the tenth hour after injection of oestrin, and increases progressively until about the twenty-fourth hour.^{5, 6, 2} In addition to these two effects, oestrin also raises the metabolic rate of uterine tissue.⁷⁻¹⁰ The present experiments were performed to ascertain to what extent the initial hyperemia, rise of metabolic activity of the tissue and motility are related to each other.

Two main groups of experiments were performed. In one, the O₂ consumption rate was measured in a group of untreated rabbits (3½ to 4 months of age), 5 of which were intact and the remainder of which (21 animals) were ovariectomized for periods ranging from 2 to 15 days. In the second group, the O₂ consumption rates were measured at intervals of 5, 10, 18, and 24 hours after a single

* Fellow, John Simon Guggenheim Memorial Foundation, resident at the University of Rochester.

¹ Markee, J. E., *Am. J. Physiol.*, 1932, **100**, 32.

² Pompen, A. W. M., *De Invloed van Menformon op der Baarmoeder*, Thesis, Amsterdam, 1932.

³ Fagin, J., and Reynolds, S. R. M., *Am. J. Physiol.*, 1936, **117**, 86.

⁴ Neumann, R., *Arch. f. Gynäkol.*, 1934, **157**, 548.

⁵ Reynolds, S. R. M., *Am. J. Physiol.*, 1931, **97**, 706.

⁶ Reynolds, S. R. M., *Physiol. Rev.*, 1937, **17**, 304.

⁷ Asscheim, S., and Gesenius, H., *Arch. f. Gynäkol.*, 1933, **153**, 434.

⁸ Khayyal, M., and Scott, C., *J. Physiol.*, 1931, **72**, 13P.

⁹ King, J. L., *Am. J. Physiol.*, 1931, **99**, 631.

¹⁰ David, J., *J. Pharm. Exp. Therap.*, 1931, **43**, 1.

intramuscular injection of 500 international units of oestradiol to rabbits ovariectomized for 9 to 11 days. The tissues were taken immediately after cerebral concussion. Each uterine horn was slit longitudinally throughout its length. Three segments were taken from each uterus, a tubal and a cervical piece from one horn and a section from the middle of the opposite horn. Oxygen consumption measurements were made in Fenn respirometers at a temperature of 37.5°C. After a 15-minute period of equilibration the chambers were closed and readings were made at 10-minute intervals throughout an hour. The hourly consumption of oxygen per milligram of dried tissue (QO_2) was determined for the various pieces of tissue dried to constant weight at 85°C.

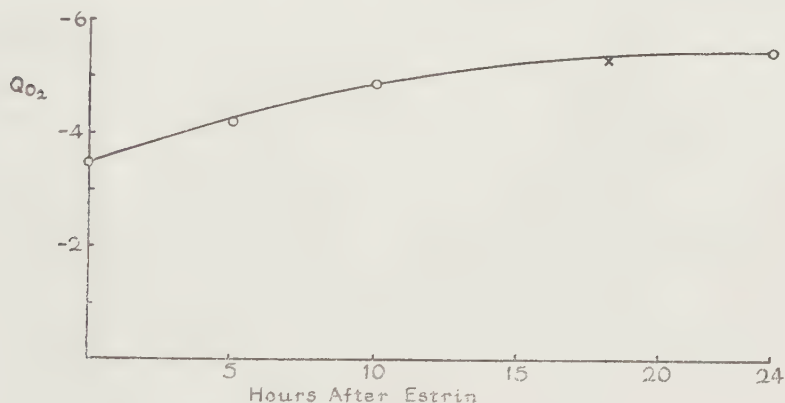


FIG. 1.

Effect of oestrin upon O_2 consumption of the uteri of rabbits castrated 10 days. Circles each indicate average of 6 animals. Cross denotes only 2 animals.

The effect of oestrin on the O_2 consumption is shown in Fig. 1. After 5 hours a rise of 20% takes place; at 10 hours it is 37%, and at 24 hours it is 60% higher than the initial level. At the end of this time the increase in O_2 consumption is about maximal, for oestrin administered as it was in these experiments. It should be noted that the time of onset of the initial hyperemia precedes any appreciable rise in metabolic activity of the tissue, and at the tenth hour, when the myometrium commences to become active, the metabolic effect is at least two-thirds complete. Clearly, therefore, the onset of rhythmic myometrial activity following the administration of oestrin depends upon a preliminary rise in the metabolic level of activity of the uterine tissue. Thus, the oestrous type of uterine motility signifies that a certain stage in the growth-response of the uterus to oestrin has been achieved.

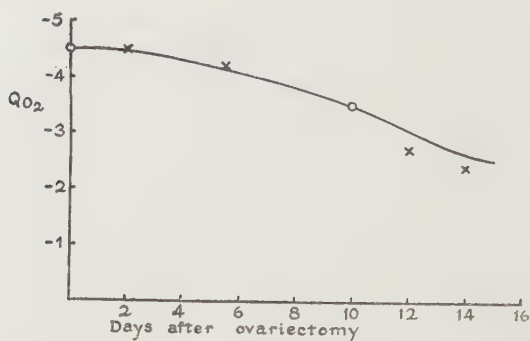


FIG. 2.

Effect of ovariectomy upon O_2 consumption of the uteri of rabbits $3\frac{1}{2}$ to 4 months of age. Five animals were used at 0 days, prior to castration, and 12 at 10 days after castration. The crosses denote only one animal in each case except at 2 days, in which 2 rabbits were used.

The effect of ovariectomy on the O_2 consumption of uteri of immature rabbits such as were used in these experiments is shown in Fig. 2. It was found that the rate of O_2 consumption was higher (28%) than was that of uteri taken from rabbits castrated for about 10 days. The removal of the ovaries at this age (and in those cases in which there are no grossly discernible graffian follicles) deprives the uterus, immature and undeveloped though it is, of a potent substance which activates the O_2 consumption of the uterine tissue. This agent is presumably oestrin.

9687

Susceptibility of Hedgehog and Agouti to Poliomyelitis.*

L. H. GROSSMAN AND S. D. KRAMER.

From the Laboratory of the Infantile Paralysis Commission of the Long Island College of Medicine.

In the present communication we wish to report the results of our continued efforts to find an animal susceptible to poliomyelitis, other than *Macacus rhesus*.¹ The hedgehog was selected because of its apparent susceptibility to a number of other viruses such as in-

* This work was supported by a grant from the President's Birthday Ball Commission for Infantile Paralysis Research.

¹ Grossman, L. H., and Kramer, S. D., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **35**, 345.

fluenza,² pseudorabies,³ yellow fever,⁴ foot-and-mouth disease,⁵ and fowl pest.⁶ The agouti, a member of the rodent family was a chance selection; it has not previously been reported on in this connection, and is readily obtainable.

On November 2, 1936, each of 3 healthy hedgehogs was inoculated into the left and right frontal lobes with 1.2 cc. of 10% centrifugalized suspension of active poliomyelitic monkey cord. Daily temperatures were taken and the animals were closely observed for changes in behavior. Two of the 3 animals died of pneumonia 7 days after inoculation. On November 7, 5 days after inoculation, the third animal was observed to drag its right hind extremity; there was no elevation in temperature. On examination of the extremity, a linear incised wound was noted. It did not appear, however, that this wound would account for the degree of disability, and the apparent flaccidness of the extremity. The animal was sacrificed. The brain and cord, which showed no gross changes, were removed *in toto* and fragments preserved in formalin for histologic study. A portion of the cord was immediately ground and 1.2 cc. of 10% centrifugalized suspension was inoculated into each of 2 healthy hedgehogs and a monkey. The monkey received an additional 2.2 cc. of the suspension intraperitoneally. One of the hedgehogs died 2 days after inoculation and the remaining hedgehog and the monkey remained unaffected.

Two additional hedgehogs were inoculated, following the technique suggested by Sawyer and Lloyd.⁷ On November 6, 1936, both animals and a Rhesus monkey were inoculated intracerebrally with 0.5 cc. of a 2% solution of boiled starch in normal saline. Approximately 7 minutes later, at the site of the previous inoculation, they received 1.2 cc. of a 10% centrifugalized suspension of virus. An additional 2.3 cc. of the suspension was inoculated intraperitoneally into each animal. One hedgehog died of pneumonia, 3 days after the inoculation; the second remained unaffected throughout the 30-day period of observation. The monkey succumbed to the experimental disease in 7 days.

On January 5, 1937, the only surviving hedgehog was reinocu-

² Stuart-Harris, C. H., *Brit. J. Exp. Path.*, 1936, **17**, 324.

³ Remlinger, P., and Bailly, J., *C. R. Soc. Biol. Paris*, 1933, **113**, 557.

⁴ Findlay, G. M., and Clarke, L. M., *Trans. R. Soc. Trop. Med. Hyg.*, 1934, **28**, 335.

⁵ Minett, F. C., 2nd Progress Report Foot and Mouth Disease Committee, London, 1927, 18.

⁶ Findlay, G. M., and Mackenzie, R. D., *Brit. J. Exp. Path.*, 1937, **18**, 258.

⁷ Sawyer, W. A., and Lloyd, Wray, *J. Exp. Med.*, 1931, **54**, 533.

lated with a 5% centrifugalized suspension of cord (1.2 cc. intracerebrally, 3.2 cc. intraperitoneally). The hedgehog remained unaffected, whereas the control monkey inoculated intracerebrally with a small infective dose of virus, succumbed to the experimental disease in 7 days.

It was our opinion, that in spite of the care given these animals, the approaching hibernating period contributed to the many fatalities. For this reason the work was discontinued until June, 1937, by which time the hedgehog is out of hibernation.

On June 8, each of 2 healthy hedgehogs and a monkey were inoculated with a 10% centrifugalized suspension of active cord in the following manner: 1 cc. intracerebrally, 2.1 cc. intraperitoneally, and on 3 successive days each animal received 1 cc. of a 10% suspension of cord into each nostril. Both hedgehogs remained completely unaffected by these treatments. The monkey succumbed to the experimental disease in 6 days.

On July 2, each of 2 healthy hedgehogs received 1 cc. intracerebrally and 2 cc. intraperitoneally of a specially prepared purified concentrate of active virus. The virus was purified by the method suggested by Schaeffer and Brebner⁸ and concentrated in the frozen state by the method devised in our laboratory and reported elsewhere.⁹ The equivalent of 100 cc. of a 10% suspension of virus was reduced to a volume of 13 cc. Both of the hedgehogs remained well over a 60-day period of observation. A control Rhesus monkey inoculated with 1 cc. of this purified concentrate, succumbed to the experimental disease in 5 days.

On June 8, 1937, each of 4 healthy agoutis and a monkey were inoculated with a 10% suspension of active poliomyelitic cord, 1 cc. intracerebrally, 2.6 cc. intraperitoneally; all 4 animals received intranasal instillations of the virus suspension, 1 cc. into each nostril, on 3 successive days. Daily temperatures were taken, and the animals were closely observed. The 4 agoutis remained unaffected by the inoculations, while the monkey succumbed to the experimental disease in 5 days. On July 3, 1937, 2 of the 4 animals and a monkey were reinoculated with the purified concentrated virus, described above; 1 cc. each intracerebrally and 2 cc. each intraperitoneally. Both agoutis remained well, while the monkey succumbed in 5 days.

Summary and Conclusions. Employing heavy suspensions of

⁸ Schaeffer, M., and Brebner, W. B., *Arch. Path.*, 1933, **15**, 221.

⁹ Kramer, S. D., Sobel, A. E., Grossman, L. H., and Hoskwith, B., *J. Exp. Med.*, 1936, **64**, 173.

active poliomyelitic cord as well as purified concentrates of these suspensions, we have been unsuccessful in transmitting poliomyelitis to the hedgehog or the agouti.

9688

Observations upon the Complement-Fixation Test in Monkeys Infected with *Endamoeba histolytica*.

CHARLES F. CRAIG AND JOHN CLYDE SWARTZWELDER.

From the Department of Tropical Medicine, School of Medicine, Tulane University of Louisiana, New Orleans, La.

Meleney and Frye¹ reported that "*Macacus rhesus* monkeys having natural intestinal infections with *E. histolytica* or experimentally infected with a human strain did not give complement fixation reactions, with one exception, and showed no macroscopic or microscopic lesions at autopsy."

During routine work in our Laboratory of Parasitology it was noted that a monkey infected with a human strain of *E. histolytica* and with *Schistosoma mansoni* consistently gave a 4-plus complement-fixation reaction for amebiasis. Because of this, and the negative results obtained by Meleney and Frye, it was thought that further observations upon this subject were indicated.

In a shipment from New York of 4 *M. rhesus* monkeys, one of the animals was found to have a natural infection with *E. histolytica*, and this strain was employed for the inoculation of the 3 other monkeys. This strain, from the naturally infected monkey, was identical in morphology with strains obtained from man; when cysts of this strain were fed to a dog an infection followed and, at necropsy, typical amebic lesions were present in the cecum and colon, containing trophozoites of *E. histolytica*, thus demonstrating that the monkey-strain was pathogenic for the dog. All of the experimental monkeys were fed concentrations of cysts from the naturally infected monkey, and all became infected and gave a positive complement-fixation reaction for amebiasis, employing an antigen prepared from cultures of a human strain of *E. histolytica*. The results of the complement-fixation tests are given in Table I.

The results shown in this table demonstrate that all of the monkeys gave a positive reaction with the complement-fixation test for

¹ Meleney, H. E., and Frye, W. W., *Am. J. Pub. Health*, 1937, **27**, 505.

672 COMPLEMENT-FIXATION IN AMEBIASIS IN MONKEYS

TABLE I.
Results of Complement-Fixation Test for Amebiasis in *Macacus rhesus* Monkeys.

Monkey	Date of Tests, 1937	Result of Tests	Remarks
MA-1			
Naturally infected with <i>E. histolytica</i>	1/5 1/20 1/27 2/11 3/4	Negative ++++ ++++ ++++ ++++	This monkey was found infected 1/6/37. Died 4/17/37
MA-2			
Experimentally inoculated 1/28, by feeding cysts of <i>E. histolytica</i>	1/5 1/27 2/11 3/4	Negative " ++++ ++++	Sacrificed 3/6/37
MA-3			
Experimentally inoculated 1/28, by feeding cysts of <i>E. histolytica</i>	1/5 1/27 2/11 2/18 3/12 4/1 4/22	Negative " +— +— +++* +++* ++	Died 4/19/37 of tuber- culosis
MA-4			
Experimentally inoculated 1/28, by feeding cysts of <i>E. histolytica</i>	1/5 1/27 2/11 3/12 4/1 4/19	Negative " " +++* +++* ++	Sacrificed 4/19/37

*A 3-plus reaction with this test is considered a positive reaction.

TABLE II.
Complement-Fixation and Lesions Present at Necropsy in *Macacus rhesus* Monkeys.

Monkey	Date of Inoculation	Date of pos- itive stool	Date of positive complement- fixation	Necropsy Findings
MA-1	Naturally infected	1/6	1/20	Lesions of amebiasis in cecum and colon containing trophozo- ites of <i>E. histolytica</i>
MA-2	Inoculated 1/28 by feeding cysts of <i>E.</i> <i>histolytica</i>	2/23	2/11	Amebic lesions in cecum and colon con- taining trophozoites of <i>E. histolytica</i>
MA-3	Inoculated 1/28 by feeding cysts of <i>E.</i> <i>histolytica</i>	2/23	3/12	No lesions and complement - fixation doubtful at time of death
MA-4	Inoculated 1/28 by feeding cysts of <i>E.</i> <i>histolytica</i>	2/15	3/12	A few amebic lesions in cecum and colon containing trophozo- ites of <i>E. histolytica</i>

amebiasis after varying periods of time following successful inoculation by feeding.

In Table II are recorded the date of inoculation, the date of the first stool-examination positive for *E. histolytica*, the date of the first positive complement-fixation reaction, and the lesions found at necropsy in these animals.

The results shown in Table II demonstrate that in 3 of the 4 monkeys amebic lesions were found in the cecum and colon containing trophozoites of *E. histolytica*; that in all 4 monkeys the stools were positive for the ameba, and that positive complement-fixation occurred in all the animals. In Monkey MA-3 the reaction was positive 2 months before death but no amebic lesions were found at necropsy, while the complement-fixation reaction had become doubtful 27 days before death. It is probable that in this animal a spontaneous recovery from the amebic infection had occurred.

Conclusions. It is concluded that the results of these experimental infections with *E. histolytica* in *M. rhesus* monkeys demonstrate that in these animals typical lesions of amebiasis occurred in the intestine and that a positive complement-fixation reaction for amebiasis followed each experimental infection.

9689 P

Effect of Deviating Urine into the Blood Stream and Intestine of Dogs.*

WILLIAM ALLYN GEER AND LESTER R. DRAGSTEDT.

From the Department of Surgery, University of Chicago.

It is now well established that elevation of the arterial blood pressure can be produced in animals by various procedures which decrease the blood flow through the kidneys.¹ A reflex effect from the ischemic kidney appears to have been ruled out as the cause of this type of hypertension by the findings that it is not prevented or relieved by denervation of the kidneys,^{2, 3} excision of the splanch-

*This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

¹ Goldblatt, Harry, Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

² Page, I. H., *Am. J. Physiol.*, 1935, **112**, 166.

³ Collins, D., *Am. J. Physiol.*, 1936, **116**, 616.

nic nerves,⁴ or complete sympathectomy.⁵ The possibility remains that a humoral agent is the responsible factor. A partial chronic reabsorption of urine was produced in 4 dogs by separating the bladder from the urethra and making a wide anastomosis between the bladder and the ileum about 12 cm. from the ileocecal valve. A definite and sustained elevation in the non-protein nitrogen of the blood occurred in each case but in all the post-operative blood pressure readings were lower than the pre-operative controls. The pressure was measured by inserting a needle connected with a mercury manometer directly into the femoral artery. Three of the animals ate very little after the operation, became progressively emaciated, and died or were destroyed in 20, 23, and 28 days respectively. One animal survived 89 days when he developed distemper and was sacrificed.

In a second series of 4 normal dogs a direct anastomosis was made between the ureter from one kidney and the lumbar vein. A glass cannula was used for this purpose and the tip was permitted to project into the lumen of the vena cava. This procedure has been reported by a number of authors^{6, 7} to cause a rapidly fatal toxemia and the existence of a specific nephrogenic toxin has been postulated to account for the result. Contrary to the experience of previous workers the animals in this series tolerated the procedure very well and there was no increase in blood pressure and no elevation in the nitrogenous constituents of the blood. The free passage of urine from the one kidney into the blood stream was indicated by the visualization of the kidney pelvis by X-rays following the intravenous injection of diodrast up to 14 days after the operation. At autopsy there was found moderate to marked hydronephrosis of the kidney on the operated side. The anastomosis was patent in 3 cases and the cannula partially obstructed by a blood clot in the fourth. In the former, slight pressure on the renal pelvis caused clear urine to flow from the cannula projecting into the vena cava. One animal died on the 13th day, the others were well when sacrificed on the 11th, 37th, and 28th days respectively.

Conclusions. (1) If the entire urinary secretion in dogs is diverted into the lower ileum, a partial reabsorption occurs, as indicated by a sustained rise in the blood N.P.N. and symptoms of toxemia, but no elevation in the arterial blood pressure is produced.

⁴ Goldblatt, Harry, *Proc. Assn. Path. and Bact.*, Boston, April 9-10, 1936.

⁵ Alpert, L., Alving, A., and Grimson, K., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **37**, 1.

⁶ Brücke, E., *Wien. klin. Wchnschr.*, 1926, **39**, 1058.

⁷ Galehr, O., and Ito, T., *Z. f. d. ges. exp. Med.*, 1927, **55**, 115.

(2) If the urine from one kidney is deviated directly into the vena cava by uniting the ureter and lumbar vein, no toxic symptoms appear, the blood N.P.N. remains within normal limits, and there is no rise in the arterial blood pressure. These experiments give no support to the theory of a specific nephrogenic toxin as postulated by Brücke.

9690 P

Effect of Cortical Hormone upon Blood Pressure in Shock Induced by Massive Doses of Adrenalin.

W. M. PARKINS, W. W. SWINGLE, A. R. TAYLOR* AND H. W. HAYS.

From the Biological Laboratory, Princeton University, Princeton, N. J.

Previous work¹ led to the conclusion that cortical hormone exerts a direct effect upon the low blood pressure in shock induced by stripping the intestine. The pressor action of the hormone appeared to be more or less independent of serum electrolyte and associated blood volume changes. The present experiments on adrenalin shock lend further support to these conclusions.

Five adrenalectomized dogs were used. They were active, vigorous, at peak weight and indistinguishable from intact animals. No hormone was administered 18 hours previous to use in the experiments. Table I shows that they had normal blood pressures, blood concentration and serum electrolytes. These dogs were given 20-40 cc. of either 1-3000 or 1-5000 solution of adrenalin (diluted with either 0.9 NaCl or 5.5% glucose) by continuous or intermittent intravenous infusion. The time interval ranged from 20-60 minutes. The animals were table trained and no anesthetic was used since the slow injection did not cause pain or discomfort. Occasionally a dog would retch or vomit if the adrenalin was given too fast. Arterial pressures were recorded by the needle puncture method² during injection and frequently thereafter. The essential data obtained from study of a representative case are given in Table I.

* E. R. Squibb & Sons Fellow.

¹ Swingle, W. W., Parkins, W. M., Taylor, A. R., and Hays, H. W., *Proc. Soc. Exp. Biol. and Med.*, 1937, **37**, 601.

² Parkins, W. M., *Am. J. Physiol.*, 1934, **107**, 518.

TABLE I.
Effect of Adrenal Cortical Hormone on Mean Arterial Blood-pressure in Adrenalin Shock.

Date	Time, hours	Blood- pressure, mm.Hg.	Pulse, min.	Serum Sodium per liter, m.-eq.	Serum Chloride per liter, m.-eq.	Serum K per liter, m.-eq.	Hemo- globin, gm. %	Red cell volume %	R.B.C., mil.	Remarks
11/18	10:00 a.m.	110	84	146.1	115.8	6.8	12.8	34.5	5.42	Normal—13.6 Kg.
"	11:15-12:15	150	60			(40 cc. adrenalin in 0.9% saline)				
"	12:18	64	128							
"	2:00 p.m.	38	108	154.7	117.8	5.8	13.2	89.7	5.88	Shock Extract* Improvement
"	3:20	55	136							H ₂ O given—no food
11/19	11:30 a.m.	72	108							Appears normal
11/22	9:30 a.m.	104	92	140.1	103.2	5.5	12.8	34.7	4.50	

*3 cc. per kg. cortical extract injected intravenously at 2:05-2:20 on 11/18/37 and 3 cc. per kg. injected in divided doses on 11/19, 20 and 21.

The arterial pressure which is high during adrenalin administration may abruptly decline to a low level shortly after cessation of injection, or the fall may be slow but steady. The animals do not show any signs of revival from shock, unless treated. They are unable to spontaneously raise the lowered blood pressure. Eight intact dogs which received approximately equivalent quantities of adrenalin per kg. body weight, given at the rate of 0.5 cc. per minute, died within 10 hours.

Intravenous injections of large doses of cortical hormone restored the profoundly shocked adrenalectomized dogs to normal health and vigor. The arterial pressure slowly rose to normal and all symptoms disappeared.

Table I shows that changes in blood concentration and serum electrolytes are negligible. The serum Na and Cl concentrations actually increased during shock whereas the K usually remained unaltered. Since loss of blood fluids to the tissue spaces is known to occur in adrenalin shocked intact dogs and cats^{3, 4, 5} some loss of vascular fluids probably occurred in our animals also. However, we do not regard such fluid loss of sufficient magnitude to adequately account for the circulatory collapse in our experiments. Likewise, the marked restorative effect of the hormone upon blood pressure appeared to be largely independent of volume changes.

It is well known that healthy adrenalectomized animals receiving maintenance doses of hormone are peculiarly sensitive to undue stress or strain of any sort, such as slight hemorrhage or mild trauma. A slowly developing, fatal shock supervenes. It is possible by utilizing these shocked dogs and injecting large amounts of potent extract, to demonstrate a direct effect of cortical hormone upon blood pressure.

³ Lamson, P. D., and Keith, N. M., *J. Pharm. Exp. Therap.*, 1915, **8**, 247.

⁴ Erlanger, J., and Gasser, H. S., *Am. J. Physiol.*, 1919, **49**, 345.

⁵ Freeman, N. E., *Am. J. Physiol.*, 1933, **103**, 185.

Skeletal Changes in Mice Receiving Estrogens.*

W. U. GARDNER AND C. A. PFEIFFER.

From the Department of Anatomy, Yale University School of Medicine.

In recent studies of the influence of estrogens on the incidence of mammary tumors in mice, comparatively large amounts of hormones have been injected over long periods of time. The appearance of lymphoid tumors in some of the experimental animals led to study of the reticulo-endothelial tissues and the discovery of extensive changes in the bones. Previous studies on the pelves of mice receiving estrogens¹ had revealed an extensive resorption of the medial and ventral parts of the pubic and ischial bones and the replacement of the pubic symphysis by an interpubic ligament.¹ Changes in other parts of the skeleton contrast markedly with those occurring in the pelvis.

Gross observations and histological studies have been made on the femurs of 34 male and female mice which had received from 100 to 1000 I.U. of hydroxy-estrin benzoate† or 0.1 mg. of equilin benzoate‡ weekly for periods up to 348 days. The remainder of the skeleton was studied by X-ray photographs. Controls consisted of 3 male and 4 female mice (uninjected) of comparable ages and one male and 2 females which had received 1/20 cc. of oil of benne (the solvent for the estrogens) weekly. The mice, which were from 4 different inbred strains,§ were all maintained under similar conditions and had access to food (Fox Chow, Ralston-Purina Co.) and water at all times.

The femurs of untreated mice and those receiving the injections of oil were a reddish color, the marrow being visible through the comparatively thin translucent compact bone of the diaphyses. Osseous spicules were found only near the epiphyses. The femurs of the animals which had received 250 I.U. or more of hydroxy-estrin benzoate or 0.1 mg. of equilin benzoate weekly for 56 or more

* This investigation has been supported by the Anna Fuller Fund and Research Fund of Yale University School of Medicine.

¹ Gardner, W. U., *Am. J. Anat.*, 1936, **59**, 459.

† The estrogens used were "Benzo-gynoestryl" obtained from the Laboratoire Français de Chimiothérapie (Paris), and Progynon-B, generously supplied by Schering Corporation through the courtesy of Drs. E. Schwenk and G. Stragnell.

‡ The equilin benzoate was obtained through Dr. G. M. Smith from Professor Girard (Paris).

§ The mice were supplied by Dr. L. C. Strong from 4 strains in his colony.

days were white, opaque, and very hard and brittle. However, unlike those of the normal mice, they were resistant to fracture at the epiphyseal lines. The marrow cavities were almost completely replaced by compact bones or by coarse bone spicules, making it impossible to insert a probe or to obtain bone marrow. Histological preparations showed that the spicules of bone filled the epiphysis, leaving only small, widely separated island of marrow. The marrow cavity of the diaphysis consisted only of a small central canal or several minute, irregular cavities containing blood vessels and a few marrow cells. The new bone seemed to be entirely endosteal in origin.

These changes are apparently reversible since in 2 cases partial recovery was observed within 2 months after the cessation of injections.

X-ray photographs indicate that the increased calcification which occurs in the femur also takes place throughout the entire skeleton, with the exception of the bones which unite at the pubic symphysis, and that certain epiphyses are closed which do not normally unite in the mouse.

Extensive studies of the bones of animals receiving estrogens have not been previously undertaken. More attention has been devoted to studies of the blood calcium, phosphorus, and phosphatase (see ² and ³), with somewhat variable results. It has, however, been reported that the epiphyses unite more rapidly in dogs receiving estrogen.⁴

Summary. Endosteal bone nearly or completely replaces the marrow cavities of the femurs of mice which have received estrogen (250 I.U. or more weekly) for long periods. Direct examination of the bones and X-ray photographs show that similar changes occur throughout the skeleton except in the immediate vicinity of the pubic symphysis.

² Folley, S. J., *Biochem. J.*, 1936, **30**, 2262.

³ Riddle, O., and Dotti, L. B., *Science*, 1936, **84**, 557.

⁴ Tausk, M., and de Fremery, P., *Acta Brevia Neerl.*, 1935, **5**, 19.

Guanidine-like Substances in the Blood of Normal and Hypertensive Dogs.

O. M. HELMER AND IRVINE H. PAGE.

From the Lilly Laboratories for Clinical Research, Indianapolis City Hospital, Indianapolis, Indiana.

Guanidine and methylguanidine when administered to dogs and cats cause the arterial blood pressure to rise probably chiefly by constricting peripheral blood vessels. It was suggested by Major and Weber^{1, 2} that they might be associated with the genesis of essential hypertension because of these properties and because they found guanidine-like substances elevated in the blood of many patients suffering from this disease. The observation of hyperguanidemia has not been generally confirmed in such cases without demonstrable renal damage.³⁻⁶ However, several investigators noted a correlation between the degree of azotemia and hyperguanidemia when retention of waste products occurred in the blood.^{8, 9} Observations of Andes, Andes, and Myers,¹⁰ and Stander¹¹ have demonstrated increased amounts of guanidine-like substances in severe toxemias of pregnancy. Minot and her associates¹² appear to have established guanidine-like substances as responsible in part for certain of the clinical manifestations of extensive tissue necrosis as in hepatic intoxication or during the morbid withdrawal of interstitial fluid into the blood stream as in diarrhea. Dodd and Minot¹³ found hyperguanidemia in several cases of tetany which exhibited insufficient changes in the inorganic constituents of the blood to account for the tetany.

¹ Major, R. H., *Am. J. Med. Sci.*, 1935, **170**, 228.

² Major, R. H., and Weber, C. J., *Bull. Johns Hopkins Hosp.*, 1927, **40**, 85; *Arch. Int. Med.*, 1927, **40**, 891.

³ Marcolongo, F., *Minerva med.*, 1933, **2**, 501.

⁴ Marcolongo, F., and Bernabo-Silorata, A., *Clin. med. ital.*, 1924, **65**, 23.

⁵ Kleeberg, P., and Schlapp, W., *Z. f. physiol. Chem.*, 1930, **188**, 81.

⁶ Bohn, H., and Schlapp, W., *Zentralbl. f. inn. Med.*, 1932, **53**, 571.

⁷ De Wesselow, O. L. V. S., and Griffith, W. J., *Brit. J. Exp. Path.*, 1932, **13**, 428.

⁸ Turries, J., *Compt. rend. Soc. biol.*, 1933, **112**, 658.

⁹ Andes, J. E., Linegar, C. R., and Myers, V. C., *J. Lab. Clin. Med.*, 1937, **22**, 1209.

¹⁰ Andes, J. E., Andes, E. J., and Myers, V. C., *J. Lab. Clin. Med.*, 1937, **23**, 9.

¹¹ Stander, H. J., *Am. J. Obs. and Gyn.*, 1932, **23**, 373.

¹² Minot, A. S., and Dodd, K., *Am. J. Dis. Children*, 1933, **46**, 522.

¹³ Dodd, K., and Minot, A. S., *Am. J. Dis. Children*, 1934, **47**, 958.

No association has been conclusively demonstrated in human beings between occurrence of increased amounts of guanidine-like substances and the appearance of hypertension. In spite of this the physiological properties of guanidine are such as to fulfill the requirements of an effector substance which has been postulated to explain the genesis of experimental hypertension in dogs.^{14, 15} For this reason the amount of these guanidine-like substances was determined in the whole blood of normal dogs and dogs made hypertensive by constricting the renal arteries by Goldblatt's clamp.¹⁶

The method of Andes and Myers¹⁷ which is a modification of those of Major and Weber² and Pfiffner and Myers¹⁸ was employed. Urea nitrogen was ascertained by the gasometric hypobromite method of Van Slyke and Kugel.¹⁹

The guanidine-like substance in the blood of 5 normal dogs varied from 0.35 mg./100 cc. to 0.74 mg./100 cc.

Unilateral nephrectomy did not alter this nor did it cause rise in

TABLE I.

Results of Determination of Guanidine-like Substances in Whole Blood of Normal and Hypertensive Dogs.

Dog No.	Blood Pressure mm. Hg.	Operation	Urea nitrogen, mg. in 100 cc.	Guanidine-like substance, mg. in 100 cc.
213	136	None	—	.42
212	140	"	—	.65
222	112	"	—	.56
223	122	"	—	.35
214	130/80	"	13.5	.74
214	136/82	Nephrectomy	—	.63
212	140/90	"	14.9	.63
209	160/100	"	28.6	.43
210	152	"	19.7	.62
216	250	1 clamp	—	.56
216	280	1 "	—	.65
189	160/100	1 "	—	.57
167	150/90	1 "	—	.56
204	242	1 "	8.8	.57
204	240	1 "	—	.79
193	252	2 "	15.1	.63
193	268	2 "	—	.57
43	286	2 "	12.6	.79
43	244	2 "	—	.72
40	224	2 "	17.8	.56
40	210	2 "	—	.65

¹⁴ Goldblatt, H., *Ann. Int. Med.*, 1937, **11**, 69.

¹⁵ Page, I. H., *Bull. N. Y. Acad. Med.*, 1937, **13**, 645.

¹⁶ Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

¹⁷ Andes, J. E., and Myers, V. C., *J. Lab. Clin. Med.*, 1937, **22**, 1147.

¹⁸ Pfiffner, J. J., and Myers, V. C., *J. Biol. Chem.*, 1930, **87**, 345.

¹⁹ Van Slyke, D. D., and Kugel, V., *J. Biol. Chem.*, 1933, **102**, 489.

blood urea nitrogen or arterial blood pressure. Constriction of either one (6 examples) or both (7 examples) renal arteries caused rise in arterial pressure to as high as 286 mm. of Hg., but neither procedure elevated the guanidine-like substances or the urea nitrogen. It is noteworthy that the blood of dogs appears to contain slightly more (0.60 mg./100 cc.) of the guanidine-like substances than human blood (average 0.24 mg./100 cc.).¹⁰

Conclusions. Guanidine-like substances exhibit physiological properties similar to those that might be expected of an effector substance liberated from the kidneys when the renal arteries are constricted by Goldblatt's clamp. Despite this, no significant increase was found in the whole blood of dogs made severely hypertensive by this method when no rise in the urea nitrogen occurred.

9693 P

A Heavily Encapsulated Form of *B. typhosus*.

T. D. BECKWITH AND H. R. MORGAN.

From the Department of Bacteriology, University of California at Los Angeles.

It has been reported by the authors¹ that if certain strains of *B. coli* are incubated within a range of temperature of 4 to 17°C., a mucoid growth will appear.

We have utilized similar technic in examining 9 strains of *B. typhosus*. All of these have been isolated recently from feces or from blood. Petri dishes containing beef-extract agar plus 1% glucose were inoculated by streaking and then were incubated at 14°C. Two of these 9 strains developed growth definitely mucoid in character but the mucilaginous consistency was less marked than that observed with the colon organism in earlier experiments.

Preparations made by Gins' India-ink method revealed long rods surrounded by heavy capsules, although parallel cultures incubated at 37°C. for 48 hours yielded the usual form of the organism which was much shorter and which was provided with much less capsular structure.

It is suggested that this form described by us may be the mucoid type of *B. typhosus*.

¹ Beckwith, T. D., and Morgan, H. R., PROC. SOC. EXP. BIOL. AND MED., 1937, 36, 606.



FIG. 1.

Heavily encapsulated form of *B. typhosus* appearing at low temperature incubation. Photograph by Dr. A. J. Salle.

9694 P

Effect of Testosterone Propionate in Eunuchoidism.

ALLAN T. KENYON, IRENE SANDIFORD, A. HUGHES BRYAN,
KATHRYN KNOWLTON AND F. C. KOCH.

From the Departments of Medicine and Biochemistry, University of Chicago.

Although the exact form and nature of the hormone secreted by the testis are unknown, testosterone, which has been extracted from bull testis, constitutes together with certain of its esters the most potent of known substances in repairing castration defects in animals. We wish to report the influence of synthetic testosterone propionate*

* We are indebted to Dr. Erwin Schwenk and Dr. Gregory Stragnell of the Schering Co. for their generous supply of testosterone propionate.

in eunuchoidism, a syndrome whose clinical expression is practically entirely that of testicular deficiency and in which it has already been shown that the excretion of androgens in the urine is materially diminished.^{1, 2}

Four eunuchoids (N.D., age 31; N.T., age 30; H.K., age 24, and J.H., age 36) received 25 mg. of testosterone propionate, intramuscularly, from 5 to 7 times weekly for 28 to 95 days. Thereafter, in the last 3, from 10 to 25 mg. 3 to 7 times weekly was given, with interruptions, for 152, 108, and 160 days respectively. In all instances there was an early increase in the frequency of erections and a marked increase in the size of the prostate (day 12 to 36). The penis elongated from 1 to 2 cm. in all but N.T. In 2 (J.H., H.K.) there was a distinct deepening of the voice (day 27-95, 26-36) and a striking development of hair in the pubic area, up the linea alba, over the thighs and on the face, while in N.T., who was further advanced to begin with, the fairly deep voice did not change and the increase in hair, while definite, was quite moderate. In J.H., nubbins of breast tissue 3x2.5 cm. in diameter appeared under the nipples (day 42) and in J.H. and H.K. a slightly opalescent fluid could be expressed from the nipples. These findings are in agreement with those of Hamilton.³

Each of these 4 men gained in weight, the amounts being for N.D. 6 kg. in 28 days, for N.T. 4.5 kg. in 41 days, for H.K. 8.5 kg. in 64 days, for J.H. 7 kg. in 42 days. An increase in appetite was reported in J.H. and H.K.; demonstrable edema was observed in J.H. and N.D.

Three eunuchoids (N.D., age 31; F.R., age 26; M.D., age 51) were studied in the hospital while on a diet constant in calories, protein, carbohydrate, fat, water and minerals, and sufficient to maintain unchanging weight. In 2 (F.R., M.D.) 3 alternate diets were used, in N.D. one diet only, served hot and cold on alternate days. Studies of the insensible weight loss on the "cold" days (N.D) by the method of Newburgh and coworkers⁴ disclosed an increase, if anything, in total calories expended during the experiment. Testosterone propionate (25 mg.) was given daily for 9 to 12 days. In all patients there was a definite increase in weight (F.R. 3.0 kg.,

¹ Gallagher, T. F., Peterson, D. H., Dorfman, R. I., Kenyon, A. T., and Koch, F. C., *J. Clin. Invest.*, 1937, **16**, 695.

² Kenyon, A. T., Gallagher, T. F., Peterson, D. H., Dorfman, R. I., and Koch, F. C., *J. Clin. Invest.*, 1937, **16**, 705.

³ Hamilton, J. B., *Endocrinol.*, 1937, **21**, 649.

⁴ Newburgh, L. H., Johnston, M. W., Lashmet, F. H., and Sheldon, J. M., *J. Nutrition*, 1937, **13**, 203.

M.D. 1.3 kg.; N.D. 1.7 kg.), in all a sharp decline in urinary nitrogen, such as described by Kochakian and Murlin⁵ in the castrate dog. This decline was reflected completely in the urinary urea (N.D.) and was unaccompanied by significant change in the nitrogenous blood constituents (N.D.). The nitrogen stored at the point of maximum weight gain (61 gm. in F.R., 23 gm. in M.D., 24 gm. in N.D.), means a storage of protein of from 144 to 381 gm., far in excess of any conceivable requirement of the growing prostate and seminal vesicles but insufficient, together with the accompanying water, to account for more than a third to a half of the total weight gain. There was in all 3 a drop in the urinary sodium and in F.R. of the urinary chlorides. The estimated sodium retention of 7.6 gm. in F.R., 5.0 gm. in M.D., and 3.8 gm. in N.D. was sufficient or more than sufficient to account, together with the associated water, for the additional weight gain and the edema observed in the longer experiments. This is in accord with the observations of Thorn and Harrop,⁶ who have induced sodium retention in the normal dog by testosterone, estrone, etc., similar to that caused by large doses of the adrenal cortical hormone. The basal metabolic rate in all 3 patients increased slightly (from 7 to 11 points) and the R.Q. in 2 experiments (F.R., M.D.) was unchanged. In one patient (F.R.) a significant creatinuria was abolished, in the other 2 creatinuria was inconspicuous. During recovery the urinary nitrogen, sodium and chloride increased and the basal metabolism declined.

Conclusions. Testosterone propionate causes enlargement of the prostate and progression of secondary sex characters in the eunuchoid, together with a gain in weight, nitrogen and sodium retention, and a slight increase in the basal metabolic rate.

⁵ Kochakian, C. D., and Murlin, J. R., *J. Nutrition*, 1935, **10**, 437; Kochakian, C. D., *Endocrinol.*, 1937, **21**, 750.

⁶ Thorn, G. W., and Harrop, G. A., *Science*, 1937, **86**, 40.

The Direct Determination of Ethyl Alcohol in Saliva without Distillation.*

THEODORE E. FRIEDEMANN. (With the technical assistance of Mr. Theodore Brook.)

From the Department of Medicine, University of Chicago.

Saliva is the most dilute of all the secretions, having a solid content of only about 0.5%.¹ It distills with less foaming, and the volatile oxidizable material, determined after a single distillation, is considerably smaller than that of blood or urine.² It contains practically no sugar and from 40 to 75% of the nitrogenous non-protein constituents usually found in the blood. Because of its relative simplicity and low organic matter content, most of the oxidizable organic matter of saliva can be removed by precipitation with $\text{CuSO}_4\text{-HgSO}_4\text{-Fe}_2(\text{SO}_4)_3$ followed by $\text{Ca}(\text{OH})_2$. Ethyl alcohol is not precipitated by this reagent and can be determined by direct oxidation of the clear supernatant solution.

No special apparatus, such as a still, is required. The precautions as to cleanliness of glassware, noted by Friedemann and Klaas² should be carefully observed.

Reagents. 1. Cu-Hg-Fe reagent. 200 cc. of water are added to 40 gm. of $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$, 40 gm. of HgSO_4 (bisulfate) and 15 gm. ferric sulfate. 25 cc. of concentrated H_2SO_4 are added and heat is applied until solution is complete. 800 cc. of water are then added. The solution is kept in a glass-stoppered bottle protected from dust.

2. $\text{Ca}(\text{OH})_2$ suspension. Ordinary unslaked lime may contain much organic matter. C. P. CaO (Merck) is therefore recommended. 100 gm. are slaked with a minimum of distilled water. 1000 cc. of water are then added. The creamy suspension is kept in a glass-stoppered bottle protected from dust.

3. Distilled Water. The precautions concerning the purity and method of storage of water noted by Friedemann and Klaas should be carefully observed.

Precipitation of Proteins, Amino-compounds, sugars, etc. 1.0

* A preliminary report of this paper was made in March, 1936, before the American Society of Biological Chemists (*Proc. Am. Soc. Biol. Chem.*, March, 1936, 37).

¹ Bodansky, M., *Introd. to Physiol. Chem.*, Wiley and Sons, New York, 2nd Ed., 1930, 147.

² Friedemann, T. E., and Klaas, R., *J. Biol. Chem.*, 1936, **115**, 47.

cc. of saliva is pipetted into a 100 cc. glass-stoppered volumetric flask. Since saliva is viscous, the pipette should be allowed to drain slowly. 10 cc. of Cu-Hg-Fe reagent and about 50 cc. of water are added. An excess of $\text{Ca}(\text{OH})_2$ is added from a pipette, and the contents are mixed by rotation. The volume is then adjusted to the mark; the contents are thoroughly mixed. The flask is allowed to stand several minutes before withdrawing a sample of the clear supernatant solution for analysis.

The ability of $\text{Cu}(\text{OH})_2$ to precipitate sugars,³ as well as the precipitation of proteins and amino-compounds by mercury,^{4, 5} has long been known. $\text{Cu}(\text{OH})_2$ also precipitates other substances. By combining the 2 reagents,⁶ a large part of the organic matter can be precipitated in one operation. $\text{Fe}(\text{OH})_3$ precipitates proteins,⁷ adsorbs many organic acids, and removes some of the sugar; its presence assures a more rapid settling of the precipitate and a clearer supernatant solution. HgSO_4 , with H_2SO_4 , is preferred to HgCl_2 because it yields a more granular precipitate.

Filtration is not recommended. It requires more glassware, it increases the number of manipulations and it introduces a variable amount of soluble oxidizable material from the filter paper.⁸ The oxidizable material appears in the first 10 to 25 cc. of filtrate; its effect on the results can be minimized by discarding this portion of the filtrate.

Oxidation. 10 cc. of the clear supernatant solution is withdrawn by means of a pipette and transferred to a carefully cleaned 150 cc. extraction flask. The latter is covered with a 100 cc. beaker. To this are added 15 cc. of water, 10 cc. of 5 N NaOH, and, with rotation, exactly 25 cc. of 0.01 N KMnO_4 . The contents are again mixed by rotation. The oxidation and final titration are carried out as directed by Friedemann and Klaas.

If speed is necessary and if only approximate results are desired, the alcohol content may be determined colorimetrically. Depending upon the alcohol content, the color changes are from violet, purple, greenish-purple, green, to brown. The colors may be compared with the colors obtained by oxidation of the supernatant solutions from pure alcohol standards.

³ Van Slyke, D. D., and Fitz, R., *J. Biol. Chem.*, 1917, **32**, 455.

⁴ Patein, G., and Dufau, E., *J. pharm. et chim.*, 1902, **15**, 221.

⁵ West, E. S., Scharles, F. H., and Peterson, V. L., *J. Biol. Chem.*, 1929, **82**, 137.

⁶ Freeman, S., and Friedemann, T. E., *J. Biol. Chem.*, 1935, **108**, 471.

⁷ Somogyi, M., *J. Biol. Chem.*, 1931, **90**, 725.

⁸ Friedemann, T. E., and Graeser, J. B., *J. Biol. Chem.*, 1933, **100**, 292.

The supernatant solution contains a considerable quantity of calcium salts which, by reaction with NaOH, lower the alkalinity. This effect is not pronounced with the 10 cc. sample recommended. However, a larger sample reduces the alkalinity below the optimum necessary for maximum oxidation in the 20-minute heating period. For larger samples more alkali should be added.

Blanks. Blank determinations with the water and reagents should be run simultaneously.

Sample Calculation. 1 cc. sample diluted to 100 cc.; 10 cc. analyzed. Blank titrations: 24.85, 24.90 cc. 0.01 *N* thiosulfate. Titration of samples: 20.37, 20.36 cc. 0.01 *N* thiosulfate. $24.88 - 20.37 = 4.51$. 1 cc. 0.01 *N* thiosulfate is equivalent to 0.042 mg. ethyl alcohol.

$$\text{Mg. \% alcohol} = \frac{4.51 \times 0.042 \times 100}{0.1} - 11 = 178 \pm 4$$

The reagent does not precipitate all of the oxidizable substances of saliva, but the residual material is small and relatively constant. Saliva was collected from 20 subjects and analyzed by the direct method as given above. The results, expressed as mg. % of ethyl alcohol, were: 11, 11, 7, 14, 8, 17, 2, 0, 9, 10, 14, 16, 13, 16, 10, 10, 5, 7, 16, 13. The arithmetic mean is 10.5, with a standard deviation of ± 4.6 . The deviation from the mean corresponds to a ± 0.1 cc. error in titration.

From 25 to 75 cc. of pure grain alcohol, diluted with water, were given to 9 subjects. Twenty-two samples were collected and analyzed by the distillation procedure of Friedemann and Klaas and by the direct method of the present paper. The results given by the 2 methods were: 142, 152; 60, 67; 66, 78; 98, 106; 89, 98; 81, 97; 98, 113; 57, 71; 50, 61; 66, 83; 63, 76; 78, 88; 64, 79; 99, 106; 103, 108; 91, 103; 69, 83; 91, 117; 94, 106; 106, 118; 95, 104; 80, 88. The alcohol concentrations varied from 50 to 142 mg. %. In every instance a higher result was obtained by the direct method. The differences varied from +5 to +26; the latter represented an unusual variation. The arithmetic mean of all determinations is 11.9, with a standard deviation of ± 4.4 . By excluding the single high result, the arithmetic mean becomes 11.2 ± 3.3 .

Summary. All but a small, but relatively constant, quantity of the oxidizable material of normal saliva may be removed by the addition of 2 reagents: (1) a solution of CuSO_4 , HgSO_4 , and ferric sulfate, and (2) a suspension of $\text{Ca}(\text{OH})_2$. The residual material

from normal saliva is equivalent to 10.5 ± 4.6 mg. % of ethyl alcohol.

A procedure for the direct determination, without distillation, of ethyl alcohol in saliva is described. The results are 11.2 ± 3.3 mg. % higher than by the method of Friedemann and Klaas.

9696

Inhibitory Effects of Male Sex Hormone on Human Menstruation and Their Evaluation by Vaginal Smears.*

GEORGE N. PAPANICOLAOU, HERBERT S. RIPLEY AND EPHRAIM SHORR.

From the Departments of Anatomy, Psychiatry, and Medicine, Cornell University Medical College, and the New York Hospital.

The suppressive effect of the secretions of the male gonads on the female sexual functions of mammals has been known for a long time. Prior to the isolation of the active principle of the testis, evidence of this effect was obtained from studies of cases of hermaphroditism, gonadal transplants, studies on freemartins, and experiments with parabiotic twins.

With the isolation of the male sex hormone, experimental evidence has been obtained of the inhibitory effect of this hormone on the sex cycle of female rodents.¹ Recently a similar effect on menstruation in primates has been demonstrated.²

The absence of comparable studies in the human has prompted this report on the effect of the male sex hormone on the menstruation of a young woman. The results obtained with the treatment of this case are in agreement with the experimental findings in animals. Furthermore, they were complemented by observations of significant morphological changes occurring in the vaginal fluid which were revealed by studies of vaginal smears.

The subject was a young woman of 18 with a history of premenstrual depression, dysmenorrhea, moderate menorrhagia, and occasional premenstrual bleeding. Physical examination was es-

* This work has been aided by grants from the Committee for Research on Sex Problems, of the National Research Council, and from the Josiah Macy, Jr., Foundation.

¹ Robson, J. M., *Proc. Soc. Exp. Biol. and Med.*, 1936, **35**, 49.

² Zuckerman, S., *Lancet*, 1937, **233**, 676; Hartman, C. G., *Proc. Soc. Exp. Biol. and Med.*, 1937, **37**, 87.

entially negative; the pelvic organs were normal. Menarche was at 13 years. The menstrual flow lasted 6-8 days and tended to be profuse. The intervals were fairly regular, usually about 30 days with an occasional variation of a few days. There was no history of amenorrhea.

Prior to treatment with male sex hormone, the patient was observed for 4 consecutive menstrual cycles, during 2 of which she received a series of injections of estradiol benzoate (Progynon-B).† This resulted in a shortening of the interval, and some diminution in dysmenorrhea. The length of these cycles and the time when vaginal smears were started are given in Chart 1.

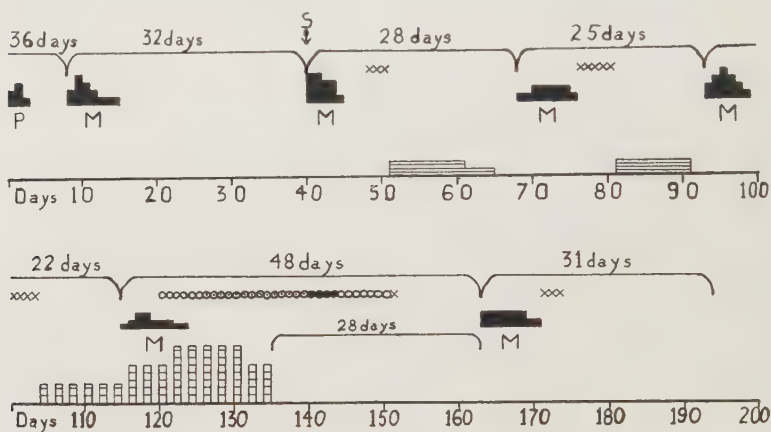


Chart I shows menstrual history and effect of treatment with estrogenic and with male hormone upon the menstrual cycle and the vaginal secretion of a young woman.

M = menstrual bleeding; one square scant, two moderate, three profuse, four excessive.

P = premenstrual bleeding. S = daily vaginal smears begun.

▬ = administration of estrogenic hormone (estradiol benzoate); each horizontal line = 500 Rat Units.

▮ = administration of male hormone (testosterone propionate); each square = 10 mg.

X = vaginal smear indicative of follicular activity. O = transitional smear types preceding and following the atrophic menopausal type. ○ = atrophic menopausal smear type.

● = menopausal smear type with abundant mucus.

CHART 1.

Beginning with the 40th day of observation, vaginal smears were taken daily. They showed somewhat subnormal follicular reactions occurring at the usual time, midmenstrually, and a fairly typical premenstrual phase with numerous bacteria resembling the *bacillus vaginalis*.

Shortly after the follicular phase, on the 12th day of the fourth

† We are indebted to Dr. E. Schwenk and Dr. G. Stragnell of the Schering Corporation for the generous supply of testosterone propionate (Oreton) and estradiol benzoate (Progynon B).

cycle, treatment was begun with intramuscular injections of testosterone propionate (Oreton).[†] During the remainder of this menstrual cycle 25 mg. of Oreton were given every other day. The next menstruation occurred on the 22nd day of the cycle and was definitely scantier. The dose was then increased to 50 mg., and later to 75 mg. every other day.

The first deviation from the normal vaginal smear was noted on the 6th or 7th day of menstruation (14-15 days from the beginning of treatment). There was a gradual replacement of the usual large squamous epithelial cell types characteristic of the normal cycle by smaller, rounder, and more compact cells with large, well-preserved nuclei.

On the 11th day (19th day of treatment) the smear consisted almost entirely of such round cells. On the 12th day of this cycle (20th day of treatment) leucocytes appeared in large numbers, and the smear became typical of the atrophic menopausal picture.³ This type of smear persisted, with slight variations throughout the course of treatment, and for 5 days after treatment was stopped. Occasional erythrocytes were present, as is common in such smears.³

On the 26th day (6 days after cessation of treatment) some increase was seen in the amount of mucus, which lasted 4 to 5 days, with an increased number of erythrocytes and fibrination. The prevailing cells were still of the "deep" menopausal type. On the 31st day of this cycle (11th day after discontinuation of treatment) leucocytes began to decrease. A few isolated cornified cells were noted on the 34th day of the cycle.

By the 37th day (17 days after treatment was stopped) a greater number of the large superficial squamous cells appeared including a few cornified cells, replacing the small "deep" cells. This indicated a resumption of ovarian follicular activity. On the 39th day, the smear was completely dominated by superficial squamous cells.

On the 40th day, the vaginal smear began to assume a premenstrual character with the reappearance of the bacillary flora originally present. It is of interest to note that this bacillary flora was absent during the induced menopausal phase. On the 48th day, exactly 28 days after the last injection of Oreton, menstruation was resumed. The flow lasted 8 days and was moderate in amount. It was followed by a weak follicular reaction, as before treatment, and then by the usual premenstrual picture, up to the next menses, which appeared on the 31st day. No masculinizing effects were observed in the patient during the period of treatment.

³ Papanicolaou, G., and Shorr, E., *Am. J. Obst. and Gyn.*, 1936, **31**, 806.

The above observations have been corroborated by similar studies in other patients in whom the smear changes have been correlated with biopsies and hormonal assays. These will be reported subsequently.

Conclusions. A study is presented in which the administration of testosterone propionate interrupted regular menstrual cycles in a young woman. Menstruation was resumed after cessation of treatment with this hormone. Vaginal smears showed that during the period of induced amenorrhea there was a replacement of the normal smear picture by one of the typical atrophic menopausal type, indicating a temporary suppression of ovarian follicular activity. This smear type was preceded and followed by characteristic intermediate stages and persisted throughout the course of treatment, and for a short time afterward. It was then followed by gradual resumption of follicular activity and a normal smear picture. The next menses occurred 28 days after the last dose of Oreton and about 12 to 13 days after the establishment of follicular activity.

This study furnishes a method for controlling the use of male sex hormone in women, and for inducing a temporary cessation of their menstrual cycle.

9697

Blood Oxygen Changes in Intermittent Claudication.

J. ROSS VEAL AND WILLIAM M. MCCORD. (Introduced by
H. H. Beard.)

From the Departments of Surgery and Biochemistry, School of Medicine, Louisiana State University, and the Charity Hospital of Louisiana, New Orleans.

Although intermittent claudication can be readily produced experimentally by obstruction of the arterial and venous circulation in a normal extremity, the pathologic state thus precipitated is widely different from the pathologic state which is seen in gradually developing arteriosclerosis. Arteriosclerotic subjects who exhibit the symptom complex of intermittent claudication present varying degrees of arterial obstruction, but no venous obstruction of any degree.

In artificially produced intermittent claudication there is a marked lowering of the oxygen content of the venous blood. In order to determine exactly what changes occur in the stage of intermittent

claudication in arteriosclerotic subjects, the following experiments were carried out on 9 patients with arteriosclerosis who presented this symptom complex, and on 9 normal subjects. The Van Slyke method was used to determine the degree of oxygen saturation.

1. In normal subjects, in the erect posture and under basal conditions, samples of venous blood were collected under oil from the lower third of the long saphenous vein and from the popliteal vein. The patient was then required to walk for 5 minutes, this period having been previously determined as the average length of time necessary to produce exercise pain in arteriosclerotic subjects. Samples of blood were collected from the same sources immediately at the conclusion of the exercise. Table I shows the percentage of oxygen saturation of the venous blood before and after exercise in normal subjects.

TABLE I.
% O₂ Saturation Venous Blood in Normal Subjects.

Cases	Rest Period		Pain Period	
	Saphenous	Popliteal	Saphenous	Popliteal
	%	%	%	%
1	71	45	79	36
2	73	40	57	28
3	28	34	57	37
4	60	32	50	27
5	52	52	63	36
6	70	37	66	23
7	40	28	36	31
8	48	48	51	34
9	58	50	48	48
Average	55.5	40.6	54	33.3

2. A similar study was then carried out in the arteriosclerotic subjects after the amount of exercise necessary to produce pain, and the duration of the pain, had been determined for each case. As in the first group of experiments, each patient stood erect for 2 to 3 minutes. Samples of blood were then collected under oil from the lower third of the long saphenous vein and from the popliteal vein, after which the patients were required to walk until the point of severe pain was reached. Samples of blood were then collected immediately from the same sources as previously. In all cases the blood was collected while the pain was still present. Table II shows the percentage of oxygen saturation of the venous blood before and after exercise in arteriosclerotic subjects with intermittent claudication.

In normal subjects the average percentage of oxygen saturation of the superficial venous blood during rest was 55.5%, as opposed

TABLE II.
% O₂ Saturation Venous Blood in Arteriosclerotic Subjects with Intermittent Claudication.

Cases	Rest Period		Pain Period	
	Saphenous %	Popliteal %	Saphenous %	Popliteal %
1	75	51	44	42
2	71	38	88	25
3	80	42	72	35
4	55	46	80	35
5	72	35	65	32
6	82	56	67	63
7	38	32	32	35
8	86	89	78	74
9	68	49	68	44
Average	69.6	49.7	66	62

to 69.6% in arteriosclerotic subjects, and after exercise was 54%, as opposed to 66.6% in arteriosclerotic subjects. In normal subjects the average percentage of oxygen saturation of the popliteal venous blood during rest was 40.6%, as opposed to 49.7% in arteriosclerotic subjects, and after exercise was 33.3%, as opposed to 42% in arteriosclerotic subjects.

Conclusions. Determinations of the oxygen saturation of the venous blood reveal a lower oxygen content in a series of normal individuals after exercise than in a series of arteriosclerotic subjects with intermittent claudication. If intermittent claudication in arteriosclerotic subjects is produced by a tissue anoxemia, it cannot be demonstrated by determinations of the oxygen content of the superficial and deep venous blood.

9698

An Improved Method for the Evaluation of Germicidal Substances.

A. J. SALLE, W. A. McOMIE, I. L. SHECHMEISTER AND D. C. FOORD.

From the Department of Bacteriology, University of California.

In previous papers^{1, 2} a new method was described for evaluating the efficiency of germicides by testing them for their effect on the

¹ Salle, A. J., and Lazarus, A. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 665.

² Salle, A. J., McOmie, W. A., and Shechmeister, I. L., *J. Bact.*, 1937, **34**, 267.

growth of living embryonic tissue and on bacteria. A number known as the toxicity-index was determined which was defined as the ratio of the highest dilution of disinfectant that showed no growth of embryonic tissue in 48 hours to the highest dilution required to kill the test-organism in 10 minutes. Theoretically the smaller the index the more nearly perfect the germicide.

The present method attempts to control several variables which were not considered in the previous work. These are: (1) performing all tests at 37°C. instead of at 25°C.; (2) keeping the time of action of germicide on tissue and bacteria the same, *viz.*, 10 minutes; (3) conducting all tests in the presence of a definite amount of organic matter.

Effect of Germicide on Tissue. Chick hearts are removed from 10-12-day-old embryos and minced into fragments about 0.5 mm. in diameter. The fragments are suspended in Tyrode's solution, about 100 pieces per cc. One-half cc. of suspension is pipetted into each of a series of tubes containing 2.5 cc. of a mixture of 3 parts embryonic extract* and 1 part defibrinated horse serum. The tubes are placed in a 37°C. waterbath.

A series of aqueous dilutions of the germicide is prepared and heated to 37°C. Two cc. of each dilution are transferred to the tubes containing the tissue-serum-extract mixture.† Final volume, 5 cc.

After 10 minutes the tubes are removed from the bath and the germicidal dilutions aspirated from the sedimented fragments. The fragments are resuspended in Tyrode's solution and the supernatant fluid again aspirated. Finally 1 cc. of Tyrode's solution is added to each tube and the fragments embedded in plasma in Carrel flasks.¹ The killing dilution is determined by the absence of proliferating cells after 72 hours or longer.

Effect of Germicide on Bacteria. One-half cc. of a 22- to 26-hr. broth culture of the test-organism is pipetted into each of a series of tubes containing 2.5 cc. of a mixture of 3 parts embryonic extract and one part horse serum. The tubes are brought to 37°C. in a waterbath.

* The extract is prepared by mincing 12-day embryos, diluting to 5 times its volume with Tyrode's solution, and centrifugating. The clear supernatant liquid is the extract.

† The germicide must be added to the mixture last since this is the order of action in clinical practice. Most germicides are considerably inactivated in the presence of organic matter. If the germicide is added to the fragments first, and then the organic material, no inactivation of the germicide will occur with the result that a smaller concentration of the chemical will produce the same effect.

A series of aqueous dilutions of germicide in tubes is also brought to 37°C. Two cc. of each dilution of germicide are transferred to the tubes containing the bacteria-serum-extract mixture. Final volume, 5 cc.

After 10 minutes the tubes are removed from the bath and a 4-mm. loopful transferred from each tube to broth. To eliminate any bacteriostatic effect subcultures are made to either 100 cc. amounts of broth or to a 15-cc. tube of broth followed by transfer of 4 loopfuls from this tube to a second one. The killing dilution is determined by the absence of growth in broth subcultures after 72 hours or longer.

Phenol, iodine, and hexylresorcinol were free from any appreciable bacteriostatic effect while the mercurial compounds metaphen, mercurochrome, and merthiolate showed a considerable effect (Table I).

TABLE I.
Bacteriostatic Effect of Some Mercurial Compounds.

Organism	Germicide	Killing concentration in 10 minutes at 37°C + organic matter		
		First transfer 15 cc. broth	Second transfer 15 cc. broth	100 cc. broth
<i>Staph. aureus</i>	Metaphen	1795	1450	1370
" "	Merthiolate	1040	25*	83
" "	Mercurochrome	13	—	13*
<i>E. typhosa</i>	Metaphen	6730	—	5665
" "	Merthiolate	3980	2660	2830
" "	Mercurochrome	175	156	140

*This concentration failed to kill all of the organisms.

Assuming that the volume of a 4-mm. loop is 0.005 cc., the final dilution of a 1:100 solution of a germicide is as follows:

$$\text{First transfer}—15/0.005 \times 100 = 1:300,000$$

$$\text{Second transfer}—15/0.02 \times 300,000 = 1:225,000,000$$

$$100 \text{ cc. broth}—100/0.005 = 1:2,000,000$$

Although the secondary transfer method gives the greatest dilution, it is subject to chance when a large percentage of the organisms are killed. Assuming 5×10^8 bacteria are added, when the final dilution is made 5×10^7 organisms would be exposed to the germicide (bacterial suspension diluted 10 times). If 99% of the organisms are killed 5×10^5 bacteria would still be viable. One loopful would contain $0.005 \times 5 \times 10^5$ or 2500 bacteria per cc. This would be added to 15 cc. of broth giving 2500/15 or 166 bacteria per cc. Four loopfuls from the first to the second tube would transfer $4 \times 0.005 \times 166$ or 3.2 bacteria. In many instances it is quite likely that no bacteria would be transferred.

In carrying out tests for germicidal activity it is necessary to distinguish between bactericidal action and bacteriostasis. The second-transfer method is more convenient to use but in the presence of a few viable cells it is not always certain that the transfer of organisms has occurred. The flasks appear to be more reliable and the dilution obtained should be sufficient to overcome any bacteriostasis.

All germicides were tested in aqueous solutions. This eliminated any germicidal effect of alcohol or other toxic solvent.

The killing concentrations of various germicidal preparations for bacteria and tissue and their toxicity-indices are given in Table II.

The order of the toxicity-indices differs with the 2 organisms, but agrees very closely with the ratings obtained by the original method.²

TABLE II.
Killing Concentrations of Germicides for Tissue and Bacteria and Their Toxicity Indices in 10 Minutes at 37°C.†

Germicide	Tissue (A)	<i>Staph. aureus</i> (B)	T.I. (A/B)	<i>E. typhosa</i> (B)	T.I. (A/B)
Iodine (Lugol's solution)	1:650	1:3520	0.2	1:3370	0.2
Hexylresorcinol	1:1450	1:1620	0.9	1:1770	0.8
Metaphen	1:2070	1:1370	1.5	1:5665	0.4
Phenol	1:224	1:110	2.0	1:186	1.2
Mercurochrome	1:90	1:12½*	7.2+	1:140	0.6
Merthiolate	1:4220	1:25*	169+	1:2660	1.6

* Failed to kill. A more concentrated solution could not be prepared.

† All results are the average of 4 determinations.

This method aims to measure the relative efficiency of germicides which are to be used to kill bacteria in the presence of living tissue. While it is not claimed that actual conditions are duplicated, it is believed that the procedure approaches nearer an "ideal" *in vitro* method than any which has been reported. The organic matter is a mixture of serum and embryonic extract, a medium somewhat analogous to the tissue-fluid found *in vivo*. The correct proportion of organic matter cannot be known since it varies greatly with different infections and conditions. The 10-minute period of exposure is probably the maximal time of effective germicidal action on a cut surface.

Of the compounds examined by this method iodine showed the lowest toxicity-index and merthiolate the highest. Iodine (in aqueous solution) appears to be the best germicide of those tested. It combines low tissue-toxicity with high bactericidal potency against both gram+ (*Staph. aureus*) and gram— (*E. typhosa*) organisms. Of the newer organic preparations hexylresorcinol was

more effective against *Staph. aureus* (gram+) while metaphen gave a better score against *E. typhosa* (gram—).

The toxicity-index indicates the relative efficiency of germicides. It does not mean that a germicide with a low toxicity-index will be harmless to living tissue-cells at any concentration. Due consideration should be given to the killing dilutions of the germicides, in the presence of organic matter, on both tissue and bacteria. The germicides, when actually used, should be diluted to the proper strengths. It may be seen from the results that a 1:100 dilution of iodine would be more toxic to tissue than a 1:100 dilution of mercurochrome. However, if a 1:1,000 solution is used, the iodine would be harmless to tissue but still bactericidal, whereas mercurochrome, though not harmful to tissue, would exert no bactericidal effect. On the basis of the results, mercurochrome and merthiolate cannot be relied upon as germicides to kill *Staph. aureus* in the presence of organic matter as used in the above experiments. However, the two germicides rate considerably better against *E. typhosa*, mercurochrome giving a lower toxicity index than merthiolate.

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A New Method of Arterial Anastomosis for Acute Experiments Without Use of an Anticoagulant.

HERMAN KABAT. (Introduced by M. B. Visscher.)

From the Department of Physiology, University of Minnesota, Minneapolis.

In cross-circulation experiments and in acute transplantation experiments, it is often necessary to use coagulable blood because surgery is contemplated after the blood vessels have been connected. Various methods have been proposed to avoid thrombosis and assure adequate blood flow in such arterial anastomoses.¹ End-to-end anastomosis with silk sutures is satisfactory but is too time-consuming for acute experiments. Arterial anastomosis by means of Payr cannulae, the method recommended by Heymans and his co-workers,² proved unsatisfactory in our laboratory.

The method of arterial anastomosis to be described is very simple and can be performed rapidly. It has been successful every time it

¹ Markowitz, J., *Experimental Surgery*, Wm. Wood and Co., 1937.

² Heymans, C., Bouckaert, J. J., and Regniers, P., *Le Sinus Carotidien et la zone homologue cardio-aortique*, G. Doin et Cie, Paris, 1933.

was attempted and has been found to provide adequate blood flow for many hours. This method involves the use of a wire eversion frame (a, Fig. 1). One artery is pulled through the loop of the frame, is everted over the loop and then tied to the frame. The everted artery is now placed within the lumen of the second artery, which is also tied to the frame. The flowing blood touches only endothelium so that thrombosis does not occur.

The eversion frame (a, Fig. 1) is made very simply by bending a piece of piano wire. The loop of the frame over which the artery is everted, should be approximately equal in size to the diameter of the artery. The 2 arteries to be anastomosed should also have about the same diameter. The frame is about 3 cm. in length and has a small loop on the far end to which threads holding the arteries are tied.

An Eversion Frame for Acute Anastomosis of Arteries

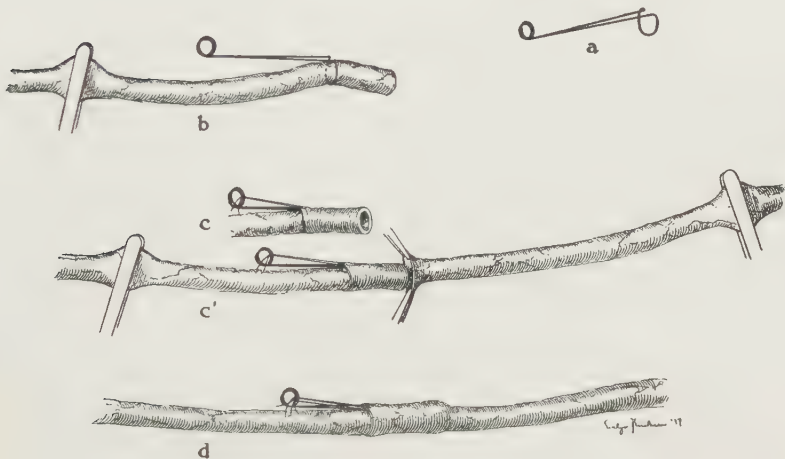


FIG. 1.

This method has been employed to connect the common carotid artery of one dog to the common carotid artery of another dog. The arteries are first exposed throughout their length and cleaned of adherent fascia. The carotid of the perfuser is clamped below and ligated high in the neck. The carotid of the perfused animal is clamped above and tied low in the neck. The artery of the perfuser is cut just below the ligature and the contained blood is milked out. A thread is then put through the wall of the artery near the cut end. The artery is then pulled through the loop of the ever-

sion frame (b, Fig. 1). With as little tension on the artery as possible, forceps are now applied and the artery is everted over the frame and the thread is tied to the far loop of the frame (c, Fig. 1). Cotton soaked in warm saline is now placed around the everted artery to protect the endothelial lining. The artery of the other dog is now cut across and a thread put through the wall near the cut end. The lumen of the artery is enlarged by means of forceps and the everted artery is placed inside of the artery of the second dog (c', Fig. 1). The outer artery is now fixed to the far loop of the eversion frame by means of the thread and the anastomosis is complete. The artery clamps are now removed and the blood flows through the anastomosis (d, Fig. 1). The pulse is practically as strong distal to the anastomosis as it is proximal to it.

It should be pointed out that there is no ligature holding the 2 arteries together. The arteries are only bound to the eversion frame and not to each other. No hemorrhage occurs, however, since the 2 arteries are held together by the arterial blood pressure. Usually a few drops of blood seep out between the 2 arteries and a small clot soon forms at the edge of the anastomosis. Since the arteries are held together only by the arterial blood pressure, it is essential that the blood flow from the everted artery into the outer artery to avoid hemorrhage.

In cross-circulation experiments using chloralose anesthesia, the carotid arteries of one dog were connected to the carotid arteries of another dog by this technic. The external jugular veins of the 2 animals were anastomosed in a somewhat different way. The cut vein of one dog was pulled through a short glass cannula, everted over it and tied to the cannula by a ligature. The everted vein was then placed inside of the vein of the other animal and this vein was also ligatured firmly to the cannula. A glass cannula is more satisfactory for venous anastomosis than is the eversion frame.

After the arteries and veins of the 2 dogs were anastomosed, the head of the perfused animal was completely isolated from its body surgically. Adequate blood flow to the isolated head was maintained for hours, since the corneal reflex was active and rhythmic respiratory movements were evident after many hours of cross-circulation. The circulation through the brain of the isolated head was adequate when only one carotid artery and one jugular vein were anastomosed to the corresponding vessels of the perfusing animal.

Capillary Pressure in Right Heart Failure.

GEORGE FAHR AND IRVING ERSHLER.

From the Department of Medicine, University of Minnesota, and Minneapolis General Hospital.

This investigation was undertaken to determine whether increased hydrostatic pressure within the capillaries was sufficient to explain the formation of edema in right heart failure without recourse to other factors such as anoxemia and increased permeability of the capillary membrane. Landis¹ has determined the gradient of intra-capillary pressure in the capillary loops of the human skin by introducing a very fine glass cannula directly into the blood capillary. He determined the pressure in the arteriolar limb, at the venous end and near the summit of the loop. Landis' method, being a direct pressure measurement, is the most accurate method for measuring capillary blood pressure. The average pressure of 125 observations in the arteriolar limb was 32 mm. Hg. and the average pressure of 99 observations in the venous limb was 12 mm. Hg., according to Landis. The average pressure from 19 observations in the summit of the loop was 20 mm. Hg. We have made determinations of the capillary blood pressure on 6 normal adults, making use of Landis' method. The values for the arteriolar limb were 30-30-30-32-35-40, or an average of 33 mm. Hg. It was only possible to make observations of the blood pressure in the summit of the loop in 2 cases. Both of these gave values of 18 mm. Hg. Measurements of the hydrostatic pressure in the venous limb of the capillaries of 6 adults gave values of 15-12-12-12-12-10 or an average of 12 mm. Hg. These values agree extremely well with the average values obtained by Landis.

The venous pressure as well as the capillary blood pressure in the venous end of the capillary loops were measured on 5 cases of right heart failure. Edema was present at the time the measurements were made. Table I contains the values observed. In a case of mitral stenosis with right heart failure and edema (Case 3, Table I) the venous pressure was 12 mm. Hg. and the capillary blood pressure in the venous limb was 25 mm. Hg. The patient became compensated, edema disappeared and another measurement showed venous pressure of 5 mm. Hg., capillary blood pressure in the venous limb 12 mm. Hg., in the middle of the loop 18 mm. Hg., and in the

¹ Landis, E. M., *Heart*, 1929-31, **15**, 209.

arteriolar limb of the capillary loop 30 mm. Hg. In all of these cases the hydrostatic pressure within the venous limb of the capillary loop was considerably higher than the mean colloid osmotic pressure of the blood, which is about 21 or 22 mm. Hg.² In one case it was found that when the patient became compensated and edema disappeared, the hydrostatic pressure within the capillary loops was normal and of such value that the passage of fluid from the capillary to the outside in the arteriolar limb would be exactly compensated by the movement of fluid into the capillary loop from the outside in the venous end of the loop. These figures are the first measurements of capillary pressure in right heart failure by the direct method. They show that it is not necessary to assume any other mechanism for the formation of edema in these 5 cases of right heart failure than an increase in the mean hydrostatic pressure along the capillaries definitely above the colloid osmotic pressure of the blood.

TABLE I.

	Venous Pressure*	Capillary Pressure*		
		Arteriolar Limb	Summit of Loop	Venous Limb
Landis' Aver. Normal Adults		32	20	12
Ersbiler's Aver. 6 Normal Adults		33	18	12
Case 1	10			35
" 2	24			41
" 3 A	12			25
B	5	30	18	12
" 4	10			25
" 5	27			35

A = edema present.

B = " absent.

* = all measurements given in mm. of Hg.

It has been assumed that dilatation of the capillaries possibly in consequence of anoxemia leads to increased permeability of the capillary wall and thus to edema formation. We have 3 observations which tend to show that dilatation of the capillary alone is not sufficient for edema formation if the intracapillary pressure is not raised. In one case of congenital heart disease the capillaries had a diameter of twice the normal size but the capillary pressure in the venous limb was 12 mm. Hg., in the middle of the loop 20 mm. Hg., and in the arteriolar limb 40 mm. Hg. No edema was present. In 2 cases of polycythemia the diameter of the capillaries was twice the normal. In one of these cases the pressure in the arteriolar limb was 35 mm. Hg. and in the venous limb 15 mm. Hg. In the other

² Kerkhof, A. C., *Ann. Int. Med.*, December, 1937, in press.

case the pressure in the arteriolar limb was 30 and the venous limb was 10. In neither of these cases was any edema present.

In one case of glomerulonephritis with a strong nephrotic tendency with edema, ascites and hydrothorax, without any heart failure but with a colloid osmotic pressure of approximately 9 mm. Hg., the capillary pressures were 30 mm. Hg. in the arterial limb and 22 mm. Hg. close to the middle of the loop. The pressure in the venous end of the capillary loop was not measured but the pressure in the median basilic vein was 3.7 mm. Hg. This shows that with normal hydrostatic pressures in capillary loops but with a lowered colloid osmotic pressure, it is possible to get edema, despite the fact that the capillaries in this case were extremely small. A diminution in the diameter of the capillary did not prevent edema formation when the colloid osmotic pressure was lower than the mean pressure along the capillary.

9701 P

Venous Pressure as a Factor in Determining Collateral Circulation in the Heart.

HARRY UNGERLEIDER, ARTHUR KERKHOF AND GEORGE FAHR.

From the Department of Medicine, University of Minnesota, and Minneapolis General Hospital.

In tying off the left descending coronary artery of the dog, it was noted by LaBree¹ that the apex was rarely infarcted if the veins were tied at the same time as the artery. Gross² showed that there was a markedly increased opening up of collateral circulation if the coronary artery of a dog was ligated after first ligating the coronary sinus. Many clinicians have noted that angina pectoris symptoms and the symptoms of coronary thrombosis are apt to be less severe in cases with severe right heart failure, *i. e.*, with a high venous pressure. With these observations in mind, we decided to ligate the left circumflex artery both with and without previous ligation of the accompanying veins and also after the production of a high pressure in the coronary sinus, one approximating the venous pressure in the coronary sinus during severe heart failure.

¹ Personal communication.

² Gross, Louis S., Blum, Lester, *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1578.

We made use of the heart-lung preparation of Starling except that the heart and the lungs were removed from the thorax of the dog and placed on a tray for greater ease of experimental approach to the coronary vessels. In 3 hearts the circumflex veins were tied first. The veins appeared to be distended and after a few moments arterial blood could be seen coming into the smaller veins. A few minutes later the circumflex artery was ligated but no infarction occurred. In one heart the main branch of the circumflex artery was tied at the same time that the veins were tied, leaving only a small branch of the artery free. Under these circumstances there was a small amount of infarction of the posterior surface of the heart. When the small free branch of the circumflex artery was tied, this infarction became complete. In 3 hearts the circumflex artery was tied without previous or concomitant ligation of the accompanying veins and in all 3 cases there was infarction of the posterior wall of the heart.

In order to produce a high venous pressure in the coronary sinus, a Morawitz cannula was passed through an opening in the right auricle into the coronary sinus where it was securely fastened. A piece of rubber tubing was connected to the Morawitz cannula from which blood flowed into a container. The height of the distal end of the rubber tubing from which the blood flowed above the right auricle determined the pressure in the coronary sinus. In one heart a pressure of 24 cm. of blood was determined in the coronary sinus by raising the outlet of the Morawitz cannula to this height above the sinus. The circumflex coronary artery was then tied and no infarction occurred. Then the pressure in the coronary sinus was reduced to that normally present and immediately infarction in the posterior surface of the left ventricle occurred. When the pressure in the coronary sinus was again raised, the infarction did not disappear. In another heart a Morawitz cannula was inserted into the coronary sinus and the pressure in the sinus raised to 20 cm. of blood. The circumflex coronary artery was then tied and an infarct resulted on the posterior surface of the heart. In another heart the pressure in the coronary sinus was raised to 32 cm. of blood by raising the height of the distal end of the rubber tubing. A ligature was then tied securely about the circumflex artery. No infarction resulted.

These experiments show that raising the pressure in the coronary veins leads to the opening up of collateral circulation. They also show that it is not necessary to ligate the veins in order to increase the collateral circulation in the area supplied by the circumflex artery

but that raising the venous pressure to about 24 cm. of blood will, at least sometimes, open up sufficient collateral circulation to prevent infarction.

9702 P

Relation Between B.M.R. and S.D.A. of Proteins.

FRANCIS M. BALDWIN AND RALPH J. SHAW.

From the Department of Zoology, University of Southern California.*

The specific dynamic action of proteins and certain amino acids has been reported to be greater than normal in conditions where a reduced basal metabolic rate prevails, *viz.*, in undernutrition¹ and fasting.² On the basis of data obtained in routine class experiments on unselected subjects, this same inverse relationship between specific dynamic action and basal metabolic rate seems to apply to persons in a presumably normal nutritional state. The subjects were 13 male and 9 female university students selected at random from a class of 40. They came to the laboratory in the early morning in a post-absorptive state and underwent the following routine:

	Min.
Rest	30
Metabolism Test†	10
Protein Meal‡	35-50
Rest	30
Metabolism Test	5
Rest	25
Metabolism Test	5
Rest	25
Metabolism Test	5

Table I gives the oxygen consumption before the protein meal and at 30, 60, and 90 minutes after. The subjects are listed in order of their relative basal rates. A comparison of the greatest percent of increase recorded at any test period with the basal rates reveals a consistently high negative relationship between the S.D.A.'s and B.M.R.'s. We have made rank-order comparisons of the data

* Acknowledgment is made to Mary L. Smull for assistance in the experiments.

¹ Mason, E. H., *J. Clin. Invest.*, 1927, **4**, 353.

² Wilhelmj, C. M., and Mann, F. C., *Am. J. Physiol.*, 1930, **93**, 69.

† Sanborn closed circuit machine.

‡ The subjects continued to recline while they were fed an amount of cooked lean beef equivalent to 200 gm. per square meter of body surface.

in Table I and find that for the male subjects the correlation§ between high B.M.R. and low S.D.A. is +.92. For the female subjects the correlation is +.80, and for the entire unselected group, +.86.

9703 P

Path of Escape of Vital Dyes from the Lymphatics into the Tissues.

PHILIP D. McMASTER AND ROBERT J. PARSONS.

From Rockefeller Institute for Medical Research, New York City.

The way in which substances of large molecule and fluids move through the tissue is unknown. To learn something of this we have studied the passage of vital dyes through living tissues, under varying physiological and pathological conditions.

Two dyes, pontamine sky blue and patent blue V, of widely different diffusibility, when introduced directly into the lymphatics of the ears of living mice^{1, 2, 3} eventually pass out in greater or less part from the channels at situations far from the site of the injection. Their escape and subsequent spread through the tissues have been watched at high magnification with the Ultropak microscope. The dyes do not pass out in a colored cloud from the lymphatics of normal ears, but first appear as very fine, irregular, bristling or wavy lines of color, extending outward from the wall of the vessel. With a micro-probe these colored lines can be bent and twisted and with the removal of pressure they spring back to their original positions. The dye appears to be fixed either between or upon preformed tissue elements. Within a few minutes of this first evidence of escape the extravascular coloration becomes more intense, the lines wider, and their margins less sharp, as though dye were diffusing from them. Manipulation still demonstrates that the dye cannot be dislodged. But soon, with the onset of the mild edema which these dyes induce in the tissues, the coloration becomes diffuse and pressure with the micro-probe modifies its intensity and forces it from

$$\S \rho = 1 - \frac{6(\Sigma d^2)}{N(N^2 - 1)}$$

¹ Hudack, S. S., and McMaster, P. D., *J. Exp. Med.*, 1932, **56**, 223.

² McMaster, P. D., and Hudack, S. S., *J. Exp. Med.*, 1932, **56**, 239.

³ McMaster, P. D., and Hudack, S. S., *J. Exp. Med.*, 1934, **60**, 479.

one region to another. It is no longer associated with the hair-like tissue elements, but has stained the edema fluid.

These are the findings in tissues previously normal. Where, however, edema had been produced prior to the introduction of the dye, by painting the ears with xylol, the character of its escape was wholly different. Now no colored, bristling lines radiated from the vessel, but the dye first appeared in a colored cloud. The color was freely movable with the micro-probe as soon as it appeared, being obviously present in the edema fluid.

When the dye, injected into normal ears, was of a sort to elicit prompt and marked edema, findings intermediate between those described were obtained. Aqueous, isotonic solutions of brom-cresol-purple (4%) and brom-thymol-blue (4%) have this effect when they are brought to pH 6.3 and 7.0 respectively. In all instances the passage of the dye from the lymph channels took the form of bristly, colored lines which could not be obliterated with pressure; but rapidly the coloration became cloud-like and freely movable, and after a minute or two the purple or blue cloud changed to buff, becoming almost invisible in the tissues. Reinjection of either indicator in the blue or purple form into the same lymphatic did not result as formerly in the appearance of discrete, bristly or wavy blue lines, but instead there appeared at once outside the channels a freely movable blue or purple cloud of dye present in free fluid.

Injections of pontamine sky blue and patent blue V into the ear lymphatics of dead mice and mice partly dehydrated by withdrawal of 40 to 50% of the blood just beforehand disclosed again the bristly and wavy lines. They developed more slowly than in normal animals but remained sharply delimited for a much longer time. In mice just injected intravenously with 1 to 1.5 cc. of Tyrode's solution, on the other hand, the bristly or wavy lines of coloration appeared seldom and transiently. There occurred instead a homogeneous coloration in the tissues.

Each of the experiments was repeated many times. The results were consistent.

What are the lines of color? Histological preparations stained for connective tissue show fine connective tissue fibers extending outward from the lymphatics in a bristling formation identical with that disclosed by the dyes in living mice. The findings strongly suggest that the dyes first extend along the interface present between the surfaces of contiguous connective tissue fibers or along that between connective tissue fibers and an interstitial gel. How-

ever this may be, it is certain that the dyes move through the tissues along tracks, as it were, determined by the form and situation of the connective tissue fibers.

9704 P

Role of Histamine in Circulatory Effects of Rattlesnake Venom (Crotalin).

CARL A. DRAGSTEDT, FRANKLIN B. MEAD AND SIMON W. EYER.

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

Essex and Markowitz¹ have made an extensive study of the physiologic action of crotalin and have pointed out a number of similarities to the action of histamine. Feldberg and Kellaway² have recently shown that crotalin has the property of liberating histamine into the perfusate when it is added to the perfusion fluid with which the lungs of cats or guinea pigs are being perfused. Their studies demonstrate a mechanism whereby the characteristic action of histamine can be superimposed upon the direct effects of the venom. The extent to which the added action of histamine influences the action of the venom in intact animals is not indicated by these experiments. In a series of studies on anaphylactic shock³ and peptone shock⁴ in the dog, we have been able to show that the degree of the vascular reaction is correlated with and dependent upon the amount of histamine liberated from the dog's tissues in these reactions. We were, therefore interested in determining whether histamine is liberated when venom is administered to the intact dog and whether the amount of histamine liberated is adequate to account for the degree of the vascular effect produced.

Crotalin* (from *Crotalus atrox*) was injected intravenously into 5 anesthetized dogs, in doses of 0.5 to 1.3 mg. per kilo, and samples of blood and thoracic duct lymph were collected during the ensuing reaction and tested for histamine. All of the reactions were severe, 2 being fatal within 20 minutes. Histamine was found

¹ Essex, H. E., and Markowitz, J., *Am. J. Physiol.*, 1930, **92**, 705.

² Feldberg, W., and Kellaway, C. H., *J. Physiol.*, 1937, **90**, 257.

³ Dragstedt, C. A., and Mead, F. B., *J. Pharm. and Exp. Therap.*, 1936, **57**, 419.

⁴ Dragstedt, C. A., and Mead, F. B., *J. Pharm. and Exp. Therap.*, 1937, **59**, 429.

* We are indebted to Mr. R. H. Hutchison of the Antivenine Institute of America for the venom used in these experiments.

in the blood in 2 experiments and also in the lymph in one of these. The identification of histamine was based upon the presence in extracts made by Code's⁵ method for the determination of histamine, of a physiologically active substance which lowered the blood pressure of the atropinized cat, contracted the guinea pig intestine, and was inactivated by diazotized sulfanilic acid. The amounts of histamine determined in the 2 positive experiments were inadequate to account for the degree of the vascular reactions, and as there were no significant quantities of histamine detected in 3 experiments, it is our belief that histamine plays only a contributory and subsidiary rôle in the vascular effects of intravenously injected venom.

Conclusions. The observation of Feldberg and Kellaway, that crotalin may liberate histamine from perfused tissues is confirmed by showing that it may also liberate histamine when injected intravenously into an intact animal. Preliminary studies indicate that the amount of histamine liberated is inadequate to account for the degree of the vascular reaction produced and that it therefore plays a contributory and subsidiary rôle to the total effect of the venom.

9705 P

Effects of Alizarine Red S Injections on the Teeth and Bones of *Macacus rhesus* Monkey.*

I. SCHOUR AND M. M. HOFFMAN.

From the Department of Dental Histology, University of Illinois College of Dentistry.

Seventeen *Macacus rhesus* monkeys of mixed or adult dentition, ranging in age from 1½ to 7 years,¹ were given multiple intravenous, intraperitoneal or subcutaneous injections of 5-10 cc. of 2% Alizarine Red S (Sodium Sulphalizarate) of color index 1034 (Coleman and Bell Company) at 5- to 30-day intervals. The time between the last injection and death ranged from 3 hours to 125 days. The dosage ranged from 40 to 60.5 mg. per kilo.

⁵ Code, C. F., *J. Physiol.*, 1937, **89**, 257.

* This investigation was aided in part by a grant from the Carnegie Corporation. The animals of this investigation were made available through the kind coöperation of Dr. George W. Bartelmez of the Anatomy Department and Dr. E. H. Lennette of the Bacteriology Department of the University of Chicago.

¹ Schultz, A. H., *Am. J. Phys. Anthropol.*, 1935, **19**, 489.

For each injection a distinct red ring was observed in the dentin and the bone that were forming and calcifying at the time.² This effect was immediate, as indicated in the animals sacrificed within a few hours after the last injection. In 6 animals 4 months elapsed between the first injection and death. This long survival did not alter the staining reaction.

Measurement of the distances between successive rings indicated the amount of apposition during the corresponding intervals. The rate of apposition of dentin based on 1164 measurements made chiefly in the ground sections of bicuspid and molars ranged from 1.6 to 5.6 μ per 24 hours. This range may be explained on the basis of a growth gradient with its maximum at the cusp and along the outer dentinal surface and its minimum apically and toward the pulp. Alizarine lines were also found in the cementum. Definite staining effects could be recognized macroscopically in the jaws and long bones.

Subcutaneous administrations were found to be complicated by a sloughing of the tissues at the site of injection. Intravenous injections gave sharper effects than intraperitoneal injections of the same dose. However, thrombosis of the veins at or near the site of injections occurred frequently. The intraperitoneal injections were as effective as the intravenous ones when the dosage was increased to 10 cc.

Preliminary experiments on rats show that intraperitoneal injections of 1 cc. of a sterile isotonic 2% solution of Alizarine Red S altered with isotonic sodium hydroxide to take effect in mammalian blood give sharp effects with little or no toxic complications and no superficial lesions. It is suggested that the preparation of an alizarine solution which would also impart radiopaque lines in the calcifying tissue would be very useful in experimental investigations.

9706

Polypneic Panting in Anesthetized Cats.

H. W. MAGOUN. (Introduced by S. W. Ranson.)

From the Institute of Neurology, Northwestern University Medical School.

In a recent series of experiments a region of the cat's brain was found which responded to local heating by acceleration of respira-

² Schour, I., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 140.

tion, panting and in some instances by the appearance of sweat on the foot pads.¹ These experiments were performed on anesthetized animals and we wish here to consider the use of anesthesia in such work.

Six experiments were performed under light nembutal anesthesia (18 mg. per kilo) with unsatisfactory results. Respiration under nembutal is slow and heating the whole animal does not lead to polypnea and panting until an abnormally high body temperature is reached. In 6 experiments the animals were prepared under ether by the insertion of a pair of electrodes into the brains and, 3 to 4 hours later, with the animals in the waking condition, heating the brain induced good polypneic panting in 3 cases, which indicates that previous etherization is not detrimental to the response. Two cats panted at a rapid rate during recovery from the anesthesia although their rectal temperatures were only 36.2°C. and 37.6°C. respectively.

Urethane does not depress respiration like nembutal, and was employed with good results in 41 experiments in a usual dosage of 1 gm. per kilo intraperitoneally. Urethane anesthesia has the disadvantage, however, of being associated in some cats with a period of polypnea and panting which may occur at a normal body temperature with no apparent external causation.

In the 41 experiments under urethane, polypneic panting in cats with relatively low rectal temperatures was encountered as a more or less initial feature in 15 cases. Eight of these 15 animals had received supplementary ether during the operative preparation and 4 had had a heating pad on during this procedure. The effect was just as marked, however, in the animals which had not received these extra attentions, and was noted to have been present in 4 cases before the operative procedure was begun. The respiratory rate during the polypnea and panting ranged between 100 and 250 per min. The rectal temperature at which the effect was present ranged from 37.5° to 40.8°C. and in 10 of the cases was between 38.0° and 38.5°C.

After a period of time ranging from 20 minutes to an hour (in 10 of the cases between 20 and 35 min.) the panting had stopped and respiration had become stabilized at a lower rate, ranging from 24 to 112 per min. The rectal temperature at this time ranged from 36.9° to 37.9°C. and in 10 of the cases was between 37.3° and 37.8°C.

The occurrence, during recovery from ether anesthesia, of pant-

¹ Magoun, H. W., Harrison, F., Brobeck, J. R., and Ranson, S. W., research in progress.

ing in 2 cats with negligible damage to the brain necessarily raises the question as to what part the anesthetic may play in the development of decorticate panting.^{2, 3}

We are unable to offer a satisfactory explanation of the effects of ether and urethane upon the panting mechanism. It is possible that they may produce a release from inhibition or they may cause a chemical excitation. Whatever the nature of their action, it is important to bear this action in mind whenever these anesthetics are used in experiments on thermal or decorticate panting.

9707

Post-Rotatory Nystagmus Following Decerebration in Guinea Pigs with Unilateral Cerebellar Lesions.

A. R. BUCHANAN. (Introduced by S. W. Ranson.)

From the Institute of Neurology, Northwestern University Medical School.

Bauer and Leidler¹ reported that they could elicit nystagmus after removal of all parts of the cerebrum as far down as the corpora quadrigemina. Wilson and Pike² could not confirm the results of these workers. They found that, as a general rule, complete removal of the cerebral hemisphere on the side of the slow deviation abolished all except a small rotatory component of the quick phase of nystagmus, while complete removal of the cerebral hemisphere of the side toward which the quick component is directed has but little effect on the quick movement.

Ivy³ performed a series of cerebral ablations in frogs, turtles, pigeons, rabbits, kittens, cats, pups, and dogs. He found, in most of these animals, that complete decerebration did not abolish the quick component of the post-rotatory nystagmus and that there was an actual increase in the nystagmus in most cases. He considered the conclusion warranted, on the basis of his findings, that the quick component of eye nystagmus is not due to the integrity of a cerebral reflex arc, as was held by Wilson and Pike, but is a lower

² Bard, P., *Am. J. Physiol.*, 1928, **84**, 490.

³ Lilienthal, J. L., Jr., and Otenasek, F. J., *Bull. Johns Hopkins Hosp.*, 1937, **61**, 101.

¹ Bauer, J., and Leidler, R., *Monatsschr. f. Ohrenh.*, 1911, **45**, 937.

² Wilson, J. G., and Pike, F. H., *Arch. Int. Med.*, 1915, **15**, 31.

³ Ivy, A. C., *J. Comp. Neurol.*, 1919, **31**, 1.

type of reflex, over which the cerebrum exercises an inhibitory influence.

Lesions were produced in the right half of the cerebellum in 9 guinea pigs by means of an Horsley-Clarke stereotaxic instrument. The lesions involved, in varying degrees, the lingular cortex, the lateral and medial cerebellar nuclei, the ventral spino-cerebellar tract, the restiform body, the brachium pontis, the uncinate fasciculus and the flocculo-nodular fibers. The right medial vestibular nucleus was also partially destroyed in 2 animals.

Following the production of the cerebellar lesions, post-rotatory nystagmus was studied, and was found to be essentially normal in rate, duration and direction. The method utilized in rotation of the guinea pigs has been described in a previous report;⁴ rotation to right or left around the basi-occipital axis resulted in horizontal nystagmus directed (quick component) toward the opposite side or forward in the ipsilateral eye and backward in the contralateral one. Increase in the intensity of the nystagmus, as described by Bauer and Leidler following destruction in the vermis, was not observed.

As a terminal procedure, the guinea pigs were decerebrated, utilizing the transection method described by Hinsey, Ranson and McNattin.⁵ The level of the transection was through the superior colliculi dorsally and the mammillary body ventrally in 5 animals, and in the remaining 4 was through the habenular trigone dorsally and the mammillary body ventrally.

After allowing time for the animals to recover from the narcosis, rotation on the turn table was again carried out. Rotation to right or left elicited after-nystagmus in the usual direction and of normal intensity in 6 animals. In one, no nystagmus appeared following rotation to the right (side of the cerebellar lesions), and in another none was elicited by rotation to the left. The remaining animal gave a somewhat reduced response following rotation to right or left which was of indeterminate direction.

Since the cerebellar lesions did not modify the nystagmus elicited by rotation previous to decerebration and most of the animals showed normal nystagmus following decerebration, it is probable that the abnormal responses in 3 animals were due to hemorrhage in and about, or trauma to the part of the brain stem remaining.

Conclusions. Post-rotatory nystagmus is still elicitable in guinea pigs with the cerebral hemispheres and thalamus removed. Neither

⁴ Buchanan, A. R., *Laryngoscope*, in press.

⁵ Hinsey, J. C., Ranson, S. W., and McNattin, R. F., *Arch. Neurol. and Psychiat.*, 1930, **23**, 1.

the slow nor the quick components of the nystagmus were disturbed by decerebration at a level which passed through the superior colliculi dorsally and the mammillary body ventrally. The direction and intensity of the nystagmus remained within normal limits.

Lesions in the right half of the cerebellum did not modify the post-rotatory nystagmus, either alone or in conjunction with decerebration.

9708 P

Epinephrine Secretion in Hypophysectomized Dogs.

J. M. ROGOFF,* E. NOLA NIXON, GEORGE N. STEWART AND E. MARCUS.

From the Physiological Laboratory, University of Chicago.

Our investigation arises from the supposed relation of the adrenal glands to the etiology and pathology of diabetes and of hypertension. This report concerns the probable interrelated functions of the adrenals and the hypophysis, in relation to these conditions.

Anatomic changes have been found in the adrenals after hypophysectomy and in the hypophysis after adrenalectomy. The adrenal cortex undergoes marked atrophy following hypophysectomy although the medulla shares but little, if at all, in this change. Extracts of the posterior lobe of the hypophysis have been reported to be capable of inducing hyperplasia of the adrenal cortex.¹ An interrenatotrophic principle, capable of affecting the adrenal cortex, has been obtained from the anterior lobe of the hypophysis and an adrenotropic action of hypophyseal extracts, affecting the medulla, has also been reported. It has been claimed² and also denied^{3, 4} that the hypertensive action of posterior pituitary extract depends upon free circulation of blood from the adrenals.

If an adrenal medullotropic function is performed by the hypophysis, it seems probable that hypophysectomy would result in a diminished rate of liberation of epinephrine from the adrenal glands. It might also be expected to affect the production or storage of epinephrine in the adrenals, as a result of loss of the hypophyseal

* Aided by the G. N. Stewart Memorial Fund and a grant from the Rosenwald Family Association.

¹ Hallion, L., et Alquier, L., *Compt. rend. Soc. Biol.*, 1908, **65**, 5.

² Képinov, L., *Compt. rend. Soc. Biol.*, 1920, **83**, 1134.

³ Hoskins, R. G., and McPeck, C., *Am. J. Physiol.*, 1913, **32**, 241.

⁴ Houssay, B. A., *Compt. rend. Soc. Biol.*, 1921, **85**, 35.

adreno-medullotropic hormone. Houssay and Mazzocco⁵ found an increase in the epinephrine content per gram of adrenal, in hypophysectomized dogs. However, they point out that the increase is not absolute since it corresponds with the diminution of adrenal weight.

Képinov⁶ concluded that the epinephrine output from the adrenals is greater in hypophysectomized than in normal dogs. His determinations were not quantitative for epinephrine secretion; he compared the vasoconstrictor action of adrenal vein blood from normal and from hypophysectomized animals, by perfusion through the isolated ear of the rabbit (Pissemski's method), but made no determinations of the actual quantity of epinephrine in the blood or of the amounts liberated by the adrenals.

We made quantitative determinations of the rate of liberation of epinephrine from the adrenals in 21 hypophysectomized dogs. The rate of epinephrine secretion was determined by the method of Stewart and Rogoff, and in 2 of the experiments the results were confirmed by another method described recently by Rogoff.⁷

Two acute and 19 survival experiments were performed. In the acute experiments the rate of liberation of epinephrine before and after hypophysectomy was determined. No significant difference was found, the epinephrine output being the same as in normal dogs.

Of the 19 survival experiments, 10 dogs were completely hypophysectomized; in 1 animal only the anterior lobe was removed; in 4 dogs a portion of the hypophysis was left; in 2 animals the stalk was partly severed and in the remaining 2 the stalk was completely severed but the hypophysis was left resting on the sella turcica. Determination of the rate of epinephrine secretion in the different animals was made at various intervals, ranging from 2 to 162 days after hypophysectomy.

Marked atrophy of the adrenals was observed in the animals that were allowed to survive longer than about a month. It appeared that the reduction in adrenal weight reaches a certain maximum, probably corresponding to the minimum amount of cortex required by the animal plus the weight of the medulla. Regardless of the weight of the animal, in the experiments that were completed about 3 to 5 months, or more, after hypophysectomy, each adrenal weighed approximately 0.3 gm. This is about $1/2$ to $3/5$ of the average weight

⁵ Houssay, B. A., et Mazzocco, P., *Rev. de la Soc. Argent. de Biol.*, 1933, **9**, 220.

⁶ Képinov, L., *Compt. rend. Soc. Biol.*, 1936, **122**, 351.

⁷ Rogoff, J. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 441.

for normal dogs, as observed from a large series (unpublished) of animals. Obviously, the capacity of the atrophied adrenal cortex to secrete the indispensable hormone, "interrenalin," is not materially altered by hypophysectomy, since animals surviving a relatively long time show no pathognomonic evidence of adrenal insufficiency.

In most instances the rate of blood flow from the adrenals was decidedly lower than is usually found in normal dogs. This might be accounted for by the atrophic changes in the adrenals induced by hypophysectomy. The epinephrine concentration in the adrenal vein blood was correspondingly greater. This can explain the results obtained by Képinov. An increased concentration of epinephrine in the adrenal vein blood, however, does not indicate an increased rate of secretion unless the rate of blood flow from the glands is not diminished.

Our experiments lead to the conclusion that the rate of epinephrine secretion from the adrenals, in dogs, is not significantly altered by hypophysectomy. Certainly, such alterations in the epinephrine output as would correspond with amounts of epinephrine necessary to have an important influence on blood sugar or on blood pressure, did not occur.

9709

Structure and Function of the Peritoneal Funnel^{*} of the Frog,
Rana pipiens.

ROBERTS RUGH.†

From the Department of Zoology, Columbia University.

Heidenhain¹ found no trace of peritoneal funnels in the frog's kidney. Spengel² first saw these funnels in such forms as *R. temporaria*, *B. cinereus*, *D. pictus*, and *Bombinator*. He claimed that in the *Coecilia* and *Urodela* there was a connection between these

^{*} Since the term "nephrostome" refers specifically to the funnel of a nephridium, the term "peritoneal funnel" is used here to designate the adult structure described without implying its origin, or its tubular connection with either the nephrocoel or hæmocoel. In this way there need be no confusion as to the adult structure found in the *Anura* or *Urodela*.

† The author wishes to express his appreciation of the technical assistance of Mr. J. Godrich, and of the unlimited research facilities offered by the Department of Zoology, Columbia University.

¹ Heidenhain, R., *Arch. f. Mikr. Anat.*, 1874, **10**, 1.

² Spengel, J. W., *Zentrblt. f. d. med. Wissensch.*, 1875, No. 2.

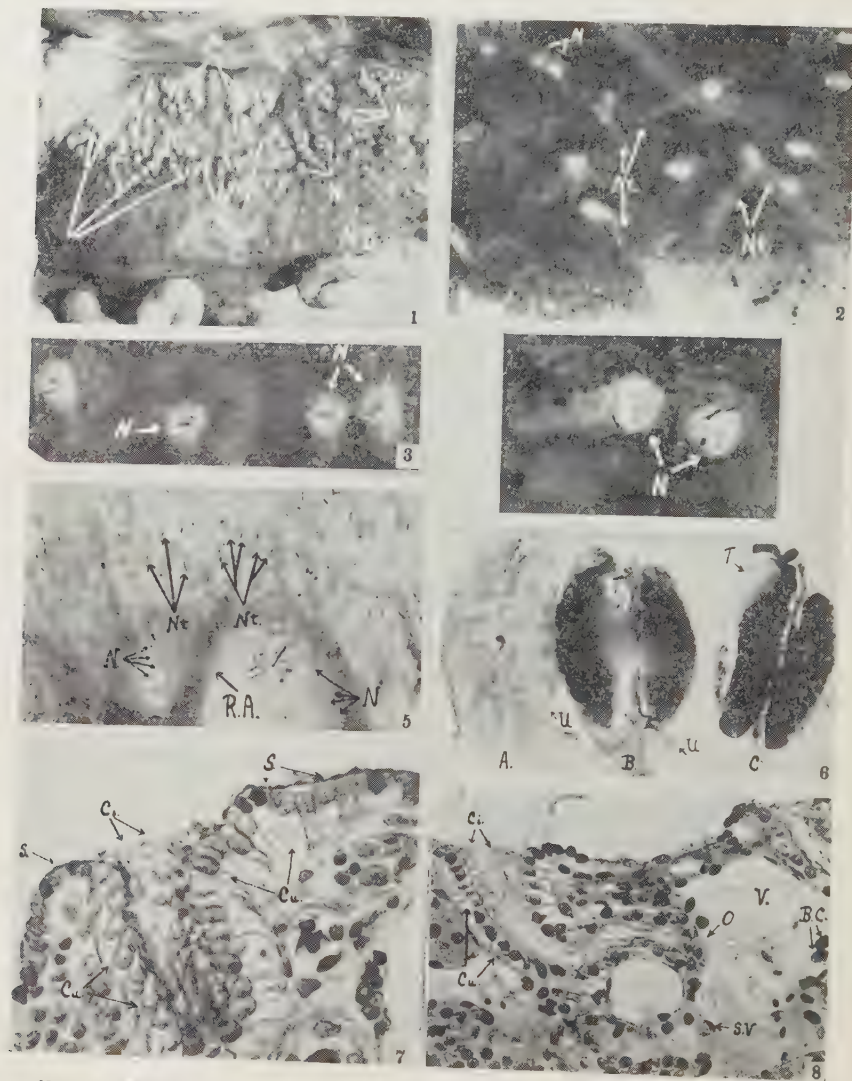


FIG. 1. Surface view of the ventral face of the kidney of an adult male frog, *Rana pipiens*, showing numerous peritoneal funnels (N); adrenal gland tissue (Ad.); and the Mullerian duct (M.D.).

FIG. 2. Enlarged view of surface of frog kidney showing peritoneal funnels (N) and related funnel tubes (Nt.).

FIGS. 3 and 4. Enlarged views of peritoneal funnels.

FIG. 5. Ventral face of frog kidney taken from frog which had been injected 5 minutes previously with a solution of india ink. Peritoneal funnel tubes (Nt.) are shown filled with ink, and numerous funnels (N) are seen clustered between the renal arteries (R.A.).

FIG. 6. Urino-genital systems excised from frogs at varying periods after the frogs had been injected with a solution of india ink. Testes (T) and Ureter (U). A. Five minutes after injection into body cavity. B. One hour after injection. C. Five hours after injection.

FIG. 7. Section through peritoneal funnel showing squamous (peritoneal) epithelium (S); cilia (Ci) within the funnel and cuboidal epithelium (Cu.) of the funnel wall, changing to columnar.

FIG. 8. Section through peritoneal funnel and tube showing connection (O) with a blood sinus (V) containing blood corpuscles (B. C.) and surrounded by typical squamous epithelium of the venous system (S.V.).

funnels and the malpighian bodies but that in *B. cinereus* he found a connection with the fourth section of the uriniferous tubule.³ Simultaneously but independently Meyer,⁴ using the silver nitrate method, counted 195 openings on the adult frog's kidney. Nussbaum⁵ reported that in larval anura the connections were between the peritoneal tubes and the neck of the malpighian body while this connection was shifted, in the adult anura, to the renal veins. He showed this renal vein connection more conclusively⁶ in *R. fusca*, *R. esculenta*, *B. calamita*, and *A. obstetricans*. In the same year Hoffman⁷ stated that these tubules in the adult anura end blindly. Ecker⁸ claimed that the funnels were very difficult to find; that they did not connect any part of the uriniferous tubule with the body cavity; and that their superficial terminations had no cilia. Marshall and Bles⁹ described the embryonic formation of these funnels and tubes and showed that in *R. temporaria* they lose their early connections with the malpighian bodies and uriniferous tubules and finally empty into renal veins. Farrington¹⁰ injected carmine into the renal portal veins and found it emerging from the peritoneal funnels but he did not indicate why blood corpuscles were not likewise liberated into the body cavity of untreated frogs. He did state that in the Urodela these tubes opened into the uriniferous tubules but in both *R. catesbiana* and *R. virescens* they opened into branches of the renal portal veins. Hall¹¹ claimed that the malpighian capsule and the developing funnel formed the short arms of a Y, the long arm of which opens into the archinephric duct, but that subsequently the funnel tube broke away and joined a blood vessel.

³ Spengel, J. W., *Arb. a. d. Zool. Zootom. Inst. der Univ. Wurtemberg*, 1876, **3**, 82.

⁴ Meyer, Fritz, *Beitrage zur Anatomie des Urogenitalsystems der Selachien und Amphibien*, Leipzig, 1875.

⁵ Nussbaum, M., *Zool. Anz.*, 1880, **3**, 514.

⁶ Nussbaum, M., *Arch. f. Mikr. Anat.*, 1886, **27**, 466; *Zool. Anz.*, 1887, **20**.

⁷ Hoffman, C. K., *Z. f. wissenschaft. Zool.*, 1886, **44**.

⁸ Ecker, A., *The Anatomy of the Frog*, translated with numerous annotations and additions by Dr. J. Haslam, Clarendon Press, Oxford, 1889.

⁹ Marshall, A. M., and Bles, E. J., *Studies from the Biological Laboratories of Owens College, Manchester*, 1890, **11**.

¹⁰ Farrington, O. C., *Trans. Conn. Acad. Sc.*, 1892, **8**, 309.

¹¹ Hall, R. W., *Bull. Mus. Comp. Anat. and Zool., Harvard*, 1904, **45**, 32.

Gray¹² showed that during the development of *R. temporaria* the lumina of the peritoneal funnels never form any connection with the lumina of the malpighian capsules but form a direct connection between the coelom and the blood system.

In *Rana pipiens* the 200-250 peritoneal funnels found on the adult male or female kidney are limited to the four-fifths of the ventral face of the kidney covered by the peritoneum (Fig. 1). The vast majority of these funnels are found clustered between the large renal arteries (Fig. 5), which are found prominently on the ventral face of the kidney. The funnel tubes are directed toward the outer margin of the kidney (Figs. 1 and 5). These tubes are connected ultimately with the posterior vena cava as will be indicated.

The peritoneal funnel openings, which have an average diameter of from 50-80 μ are highly ciliated (Figs. 7 and 8). In male frogs these are the only cilia found in the body cavity but in females the peritoneal lining is quite completely covered with cilia which are developed as a secondary sexual character (Rugh¹³). The cilia project slightly from the open end of the funnel (Fig. 7) but their constant and effective strokes are inward toward the funnel tube. The squamous (peritoneal) covering of the kidney disappears at the mouth of the funnel and beneath this epithelium, as the funnel is approached, there appears at first cuboidal and then almost columnar epithelium. The cells of the funnel and related tube bear numerous cilia, not single flagellæ as has been described for other forms. The tubule bends immediately, coursing just beneath the surface of the kidney (Figs. 2 and 8) and empties shortly into a blood sinus.

The function of these funnels and tubes can be easily demonstrated by injecting into the body cavity of each of several frogs 5 cc. of a solution of 1 part Higgins India ink and 2 parts of Holtfreter's¹⁴ modification of amphibian Ringer's solution. This injection may be made at any point into the body cavity and will in no way affect the normal activity of the frog. Within 5 minutes, at ordinary laboratory temperatures of 22-25°C., one of the frogs may be opened, the excess ink in the body cavity washed away, and the entire urino-genital system quickly excised. It will be noted under binocular magnification and direct lighting that the funnels and tubes have already taken up considerable ink (Figs. 5 and 6A), but no trace of ink will yet be found in either the blood or excretory systems. The distribution of the funnel openings and the shape

¹² Gray, P., *Quart. J. Mic. Sc.*, 1930, **73**, 507.

¹³ Rugh, R., *J. Exp. Zool.*, 1935, **71**, 163.

¹⁴ Holtfreter, J., *Arch. f. Ent. Mech. der Org.*, 1931, **124**, 404.

and direction of the tubules (Fig. 5) coincide with the observations on the untreated kidney (Fig. 1). If a second frog is similarly examined after a lapse of one hour, it will be noted (Fig. 6B) that the funnels and tubules can no longer be distinguished because the complexly arranged renal blood vessels have become filled with ink particles. Ink will be found in the posterior vena cava, in the liver lobes (which will be black), and toward the anterior part of the body generally. The white testes in Fig. 6B show, as yet, no evidence of ink within their blood vessels. However, after 5 hours (Fig. 6C) there is very little ink left in the body cavity; the kidneys, liver, heart, and lungs are blackened by an accumulation of ink within their blood vessels; and even the arteries of the testes show the presence of pigment. This means that ink particles have been carried through the heart into the arterial system. Much of the ink is retained within the kidney itself, as can be noted by comparing Figs. 6B and 6C.

That the tubule is connected with a blood sinus is also histologically evident (Fig. 8). This connection is seen to be very near the ventral surface of the kidney, a fact which can be confirmed by a study of the living kidney at various intervals after exposure to ink. A study of both dorsal and ventral surfaces of the kidney indicates that these ink particles are limited to the ventral surface. If the tubules had any connection with the excretory units (the malpighian bodies or uriniferous tubules) ink would be seen almost immediately on the dorsal surface of the kidney and in the ureters. These ureters are clear (Fig. 6). In these tests males were used exclusively because the large ovaries of the hibernating female act as partial obstruction to the peritoneal funnels and also because the ostiae could alone rid the body of foreign material (Rugh¹⁸).

Summary. The peritoneal funnels of the adult frog, *Rana pipiens*, number from 200-250 and have an average diameter of 50-80 μ ; are confined to the ventral face of the kidney covered by peritoneum; and are clustered near the inner margin of the kidney. These funnels are constantly open to the body cavity and show no variations as to sex. They are highly ciliated and carry material (liquid or particles) from the body cavity, through short tubes just beneath the surface of the kidney, into venous sinuses and hence to the posterior vena cava. In adult frogs there is no connection between these tubules and the excretory units of the kidney (malpighian corpuscles) but they are excretory in the sense that they carry peritoneal contents into the blood system whence such material may be ultimately eliminated through regular excretory channels.

A Study of the Factors Concerned in Renal Hypertension.*

L. N. KATZ, M. MENDLOWITZ† AND M. FRIEDMAN. (In collaboration with W. Weinstein and S. Rodbard.)

From the Cardiovascular Department, Michael Reese Hospital, Chicago, Ill.

Since the fundamental work of Goldblatt and his associates,¹ persistent hypertension in dogs by renal ischemia has been produced by other investigators.²⁻⁵ However its etiology and exact mechanism are still unknown.

We have been particularly interested in the effects of clamping one of the renal arteries. Various observers^{1, 5, 6} have noted that following the clamping of one renal artery, one of 3 results may be expected; *viz.*, (a) a permanent rise, (b) a temporary rise or (c) no alteration in blood pressure. This report is based on observations in 6 dogs in whom unilateral renal artery clamping by the Goldblatt technique¹ was carried out. Five of these 6 dogs showed a rise in blood pressure following this procedure. In 2 of these 5 dogs, the blood pressure returned to normal within 3 and 8 weeks, respectively; the blood pressure in the other 3 fell somewhat but did not return to the preoperative level. The blood pressures were uniformly determined in the afternoon on trained animals. A direct arterial puncture was made under local anesthesia (2% procain subcutaneously) with an ordinary hypodermic needle. The pressure was recorded with a calibrated Hamilton manometer on bromide paper.⁷ From 30 to 101 days following the unilateral clamping, the normal kidney was removed from each of the 6 dogs, leaving only the kidney with its renal artery previously clamped. In 5 of the 6 dogs, a marked rise in both systolic and diastolic pressures occurred following the removal of the normal kidney. The sixth

* Aided by the A. D. Nast Fund for Cardiac Research.

† Working under a grant from the Emanuel Libman Fellowship Fund, Mt. Sinai Hospital, New York.

¹ Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

² Collins, D., *Am. J. Physiol.*, 1936, **116**, 619.

³ Elaut, L., *Compt. rend. Soc. de Biol.*, 1936, **122**, 126.

⁴ Page, T. H., and Sweet, J. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 260.

⁵ Blalock, A., and Levy, S. E., *Ann. Surg.*, 1937, **106**, 829.

⁶ Harrison, T. R., Blalock, A., and Mason, M. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **35**, 122.

⁷ Hamilton, W. F., Brewer, J., and Brothman, I., *Am. J. Physiol.*, 1934, **107**, 427.

dog, which died in uremia and had never shown any rise in blood pressure, was found at necropsy to have had the renal artery completely occluded by the Goldblatt clamp. Three of the other animals also died in uremia, while 2 are still living. In the 3 latter dogs dying in uremia, the pressure rise following removal of the normal kidney averaged 38 mm. Hg. for the systolic and 33 mm. Hg. for the diastolic pressure. The other 2 dogs which are still living (one has now survived for 90 days following the nephrectomy) showed an even greater rise in pressure following removal of the normal kidney, *viz.*, an average of 62 mm. Hg. for the systolic and 32 mm. Hg. for the diastolic pressures.

Fig. 1 is a chart of one of the latter dogs. It will be observed that there is a marked rise in blood pressure following the removal of the normal kidney greater than the rise previously caused by partially occluding the renal artery of the other kidney. The blood non-protein nitrogen in this dog was always relatively low and at no time was there evidence of uremia. Blalock and Levy⁵ and Goldblatt⁸ also have observed a rise in blood pressure following the removal of the normal kidney in a dog whose other kidney had been rendered ischemic previously.

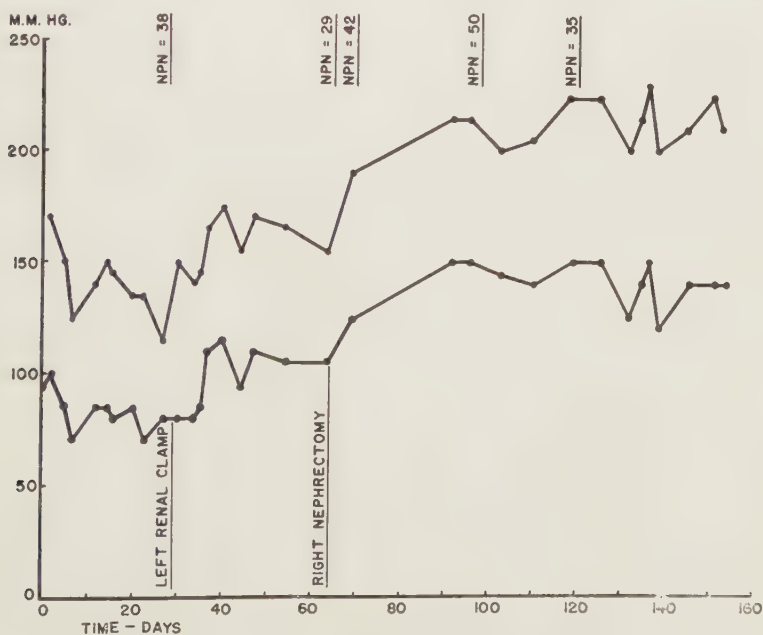


FIG. 1.
NPN is blood non-protein nitrogen in mg. %

⁸ Goldblatt, H., personal communication.

These experiments clearly indicate that the temporary hypertension produced by ischemia of a single kidney may reappear or be further increased and protracted when the remaining normal kidney is removed. Experiments such as ours are difficult to reconcile with a neurogenic origin of renal hypertension but fit better with the current view of a humoral origin. It is noteworthy that removal of a normal kidney not only permits the development of hypertension, but sometimes of uremia as well. Hypertension of renal origin depends, our results suggest, on the ratio of ischemic to normal kidney tissue rather than on the amount of ischemic renal tissue alone. Decreasing the former will tend to have an effect similar to increasing the latter.

Summary. Six dogs had ischemia produced in one kidney by a Goldblatt clamp applied to one renal artery, and after a varying time interval, the normal kidney was removed. This latter procedure caused a noticeable rise in blood pressure in 5 out of the 6 animals, and 4 of the animals died in uremia.

9711 P

Treatment of Hepatomegaly in Juvenile Diabetes Mellitus with a Pancreatic Extract.

HAROLD G. GRAYZEL AND LEO S. RADWIN. (Introduced by B. Kramer.)

From the Department of Pediatrics and the Pediatric Research Laboratories of the Jewish Hospital of Brooklyn.

Hepatic enlargement is not a common complication of diabetes mellitus. Its incidence is much higher during the first 2 decades of life than later.¹ Excluding cirrhosis of the liver, the most frequent pathological finding in cases of this type is a fatty infiltration and degeneration of the liver.

As a rule the condition occurs in severe and uncontrolled diabetes.^{1, 2} In most cases, adequate management of the diabetes with diet and insulin, and more recently with protamine-zinc-insulin^{1, 2} results in hepatic recession. However, there are instances where careful dietary management coupled with adequate insulin therapy

¹ Hanssen, P., *J. A. M. A.*, 1936, **106**, 914.

² White, P., *Diabetes in Childhood and Adolescence*, Philadelphia, Lea and Febiger, 1932, p. 169.

fails to accomplish a return of the liver to its normal size. We have in our own clinic 4 patients whose livers have enlarged progressively in spite of such treatment.

In animal experimentation, similar experiences have been encountered. It has been observed that completely depancreatized dogs, even though they were treated with insulin, showed extensive fatty infiltration and degeneration of the liver. The changes did not occur, however, if the dietetic and insulin therapy were supplemented by the feeding of raw whole pancreas.^{3, 4}

The administration of adequate amounts of lecithin or choline to the animals' diet also succeeded in preventing the development of these pathological changes.⁵⁻⁹ However, Ralli, *et al.*,¹⁰ have demonstrated that the feeding of raw pancreas is more effective in accomplishing this end than is the addition of lecithin or choline. Van Prohaska, *et al.*,¹¹ showed that the fatty infiltration and degeneration in the liver is not due to the absence of pancreatic juice from the alimentary tract, and that lecithin or choline are probably not the active principles in raw pancreas which are concerned in preventing these changes. The same workers⁴ have produced an alcoholic extract of beef pancreas, the administration of which to depancreatized dogs succeeded in preventing the appearance of fatty changes in the liver or in causing their disappearance where already present. Upon the basis of their work in animals, we undertook a determination of the efficacy of such an extract in 3 of our diabetic patients with hepatomegaly.

Three diabetic children, who for a number of years had suffered with extensive enlargement of the liver, were selected. Previous efforts to cause a reduction in the size of their livers had all been unsuccessful. They were now treated with a pancreatic extract prepared according to the method of Dragstedt, *et al.*⁴ During the course of the investigation, blood lipid studies were performed at intervals. At first the pancreatic extract in saline solution was ad-

³ Allan, F. N., Bowie, J. J., Macleod, J. J. R., and Robinson, W. L., *Brit. J. Exp. Path.*, 1934, **5**, 75.

⁴ Dragstedt, L. R., Van Prohaska, J., and Harms, H. P., *Am. J. Physiol.*, 1936, **117**, 175.

⁵ Hershey, J. M., and Soskin, S., *Am. J. Physiol.*, 1931, **98**, 74.

⁶ Best, C. H., and Hershey, J. M., *J. Physiol.*, 1932, **75**, 49.

⁷ Best, C. H., and Huntsman, M. E., *J. Physiol.*, 1932, **75**, 405.

⁸ Best, C. H., Ferguson, G. C., and Hershey, J. N., *J. Physiol.*, 1933, **79**, 94.

⁹ Best, C. H., and Channon, H. J., *Biochem. J.*, 1935, **29**, 2651.

¹⁰ Ralli, E. P., Flaum, G., and Banta, R. J., *Am. J. Physiol.*, 1936, **110**, 545.

¹¹ Van Prohaska, J., Dragstedt, L. R., and Harms, H. P., *Am. J. Physiol.*, 1936, **117**, 166.

ministered to the 3 subjects. Because it was not palatable in this form, and its ingestion caused nausea, it was discontinued. It was subsequently desiccated and given again in salol coated capsules.

Where the extract was taken regularly, there was effected a reduction in the size of the liver to normal within from 3 to 5 months. Upon discontinuing the medication the enlargement of the liver recurred in 1-2 months. In each case the decrease in the size of the liver was accompanied by a significant lowering of the total serum lipids and lipid phosphorus.

One can reasonably assume that the introduction of the specially prepared pancreatic extract given in addition to diet and insulin was responsible for the recession of the liver in these 3 juvenile diabetics, where careful control of diet and adequate insulin treatment had been unsuccessful.

9712

Suitability of Inulin for Intravenous Administration to Man.

HOMER W. SMITH, HERBERT CHASIS AND HILMERT A. RANGES.

From the Departments of Physiology and Medicine, New York University College of Medicine, and the Third (New York University) Division of Bellevue Hospital.

Attention has been called to the fact that, while some samples of inulin are physiologically innocuous, others, apparently of the highest chemical purity, may induce febrile reactions, accompanied in the more severe forms by chills, nausea and lumbar pain.¹ According to Co Tui, *et al.*,^{2, 3, 4} the pyrogenic activity can be removed by passing the solution through a Seitz serum filter or EK filter, a fact which we have confirmed for the EK filter. Treatment with filter material (asbestos) in bulk is ineffective. Originally we removed the pyrogenic activity as follows: 750 gm. of dry inulin (8% water) are dissolved in 1500 cc. of hot distilled water and boiled for 5 minutes with 50 gm. of Norit A; the solution is filtered hot

¹ Goldring, W., and Smith, H. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 67.

² Co Tui, McCloskey, K. L., Schrift, M. H., and Yates, A. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **35**, 297.

³ Co Tui, Schrift, M. H., McCloskey, K. L., and Yates, A. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 227.

⁴ Co Tui, McCloskey, K. L., Schrift, M., and Yates, A. L., *J. Am. Med. Assn.*, 1937, **109**, 250.

with the aid of suction, mixed with an equal volume of 95% alcohol, and chilled in the icebox. The inulin is filtered out by suction and well drained by hand pressure. If further purification is desired the moist inulin is dissolved in 1200 cc. of distilled water and the above process repeated; otherwise it is dried at 50° for 15 to 24 hours. This method was used by the Pfanstiehl Chemical Company to purify inulin on a large scale, the resulting preparations being non-reactive in man in doses of 100 gm., administered intravenously as a 20% solution in 1.0% NaCl, injected at a rate of 6 to 10 cc. per minute. But more recently Norit extraction has failed to work, both in our hands and in the Pfanstiehl laboratory, and until the method is corrected we are removing the reactivity by filtering the 5 or 10% solution through a Seitz EK serum filter.

Preliminary tests may be made upon dogs by giving the inulin intravenously in doses of 0.5 to 2.0 gm. per kg.; powerfully reactive inulin produces fever of 104 to 105°, usually accompanied by weakness, tremors, vomiting and diarrhea one to 4 hours later, while less reactive preparations produce only slight degrees of fever. It is as yet uncertain that the absence of a reaction in the dog is certification of the innocuousness of the material in man, and we prefer to examine every new lot in man by starting with small doses. The inulin is dissolved in hot, sterile saline, filtered, boiled, and administered by syringe or infusion in from 5 to 20% solution. Testing is begun with a 0.5 gm. dose and if negative the dose is doubled until 100 gm. are given. Hourly rectal temperatures are observed for 6 hours after the injection. Temperatures between 100 and 100.5° are suspect, while rectal temperature above 100.5° or definite subjective complaints are indicative of pyrogenic activity.

Tolerance to inulin reactions can be developed in both man and dogs, as shown by the following: 2 volunteer subjects received 2.0 gm. of inulin No. 684 (a highly reactive preparation) intravenously and both showed a reaction in the form of a severe headache, a chill lasting 20 minutes, increase in pulse rate, a temperature above 102° and perspiration; this dose was repeated on the second day with a diminution of symptoms, and again on the third day with no reaction other than a slight rise in temperature (101 and 100.4°); a fourth injection on the fifth day was again negative, the highest temperature being 100 and 100.4° with no subjective symptoms whatever. Two dogs injected with No. 684 and a third with No. 1382 (0.5 gm. per kg.) showed a marked reaction evidenced by vomiting, defecation, depression and fever. On successive identical injections, 2, 4, 6, 8, and 10 days later the reactions,

including fever, disappeared entirely. Five weeks after the last injection the same samples in the same dose again produced fever, vomiting, tremors and depression, indicating that tolerance had disappeared.

Since our previous report (*loc. cit.*) and prior to the introduction of the Seitz filter method, we have administered intravenous infusions of chicory or dahlia inulin in doses exceeding 20 gm., 168 times. Usually a priming infusion of 100 cc. of saline plus 15 gm. of inulin was given at the rate of 10 cc. per minute, followed by the slow infusion of 5% inulin in saline. The approximate total dosage of inulin has ranged as follows: 20 to 29 gm., 58 times; 30 to 39 gm., 50 times; 40 to 49 gm., 44 times; 50 to 80 gm., 12 times; 100 gm., 3 times. Excluding a few instances of mild afternoon headache, subjective complaints indicative of pyrogenic reaction have been made in 11 instances. In one instance the reaction was attributable to the saline; in a second, the reaction (chills and a slight fever) appeared to be due to urethral trauma; in 4 instances it is believed that the reaction was due to the administration of unduly warm infusion fluid, a circumstance that arose when we were not using a thermometer to check the cooling coil in the infusion apparatus, and it was possible for excessively warm solution to enter the arm.

The remaining 5 reactions were probably attributable to inulin. One of these consisted of a severe headache, but the maximum afternoon temperature was normal (99.6). Two subjects complained of abdominal-lumbar pain during or shortly after rapid infusion (10 cc. per minute), the pain disappearing when the infusion was stopped for a short time, and not recurring when the infusion was continued at a slower rate. The maximum afternoon temperature of these 2 subjects was also normal (99.2). The 2 remaining subjects showed an unmistakable reaction, as evidenced by chill, marked subjective complaints and fever, the afternoon temperature rising from 102 to 103°; it should be noted, however, that the same lot of inulin which induced these 2 reactions was administered 86 times without reaction to other subjects, some before and some after the time when these 2 reactions were observed.

In view of our inability to account otherwise for the 2 distinctly febrile reactions and the other instances of mild complaint recorded above, we infer that some subjects may show an unmistakable reaction with samples of inulin that produce no reaction in other individuals. However, we believe that if the material has been adequately tested untoward reactions accompanying the use of physio-

logically tested inulin are no more frequent than with other intravenous infusions of like duration. Scrupulous care must be observed with respect to the saline and the infusion apparatus. From experience with over 250 infusions carefully observed for reaction, we place considerable confidence in Sterisol saline bottled in pyrex ampoules. The rubber tubing, before being put into use, should be thoroughly cleaned by filling it with N NaOH, stoppering the ends and rolling it vigorously so that the lumen is cleaned by friction, which process removes astonishingly large quantities of particulate material from the best "intravenous" clear gum rubber tubing. It is then rinsed and sterilized by boiling. It must be washed at once and with particular care after use with inulin solutions to prevent the inulin from crystallizing out and adhering to the walls or in small crevices, since these slight incrustations may be a source of reaction.

9713 P

Hemodynamic Effects of Subcutaneous, Submucosal, and Subgingival Injections of Procaine-Epinephrine Hydrochloride Solutions.

PAUL P. PICKERING, H. P. STEINMEYER AND ARNO B. LUCKHARDT.

From the Department of Physiology and Walter G. Zoller Memorial Dental Clinic, University of Chicago.

The investigations of Luckhardt and Koppanyi¹ were concerned with the pressor effects of epinephrine hydrochloride injected subcutaneously and the effect of massage thereon. In the present work, two problems and their ramifications have been and are being studied. First, the absorption of epinephrine from injections in subcutaneous areas as compared with submucosal injections in the oral cavity. Secondly, the effect of subgingival injections of procaine-epinephrine hydrochloride solutions. Conclusions were drawn from a comparative measurement of the blood pressure changes as evidenced from these injections.

Dogs, 38 in number, were used in the series of experiments. An area on the chest wall was selected for the subcutaneous injections, whereas a site in the dog's mouth at the mucobuccal fold offered an easily accessible area for the submucosal injections. In some of the

¹ Luckhardt, Arno B., and Koppanyi, Theodor, *Am. J. Physiol.*, 1927, **81**, 436.

animals the subcutaneous injections were made prior to the submucous injections, whereas on others the order of procedure was reversed to avoid any misinterpretation of results from the influence of previous epinephrine deposits.

Following submucosal injections, a rise in blood pressure ranging between 10-40 mm. of Hg. was noted. These rises were greater than those recorded from similar injections in the subcutaneous tissue, the greatest being 10 mm. of Hg. The time required for the blood pressure to return to a normal level was slightly greater following submucosal injections.

Following the technique described by Luckhardt and Koppányi¹ and Koppányi and Luckhardt,² the areas of injection were massaged lightly. The massage of the 2 sites resulted in a greater pressor response from the submucous injection.

The second series of experiments was concerned with the pressor responses from various concentrations of procaine-epinephrine deposited in several selected sites in the mouth of the dog.

The procaine (2%)-epinephrine solutions ranged in epinephrine concentration from 1/10,000 to 1/30,000, all injections having a volume of 2 cc. regardless of site of injection or size of dog.

Several areas for deposition of the solution were selected, namely: (1) In the region of the posterior palatine foramen (Fig. 1A). (2) Into the mental foramen of the mandible (Fig. 1B). (3) In the region of the mandibular foramen (Fig. 1C). (4) Into the anterior palatine foramen (Fig. 1D).

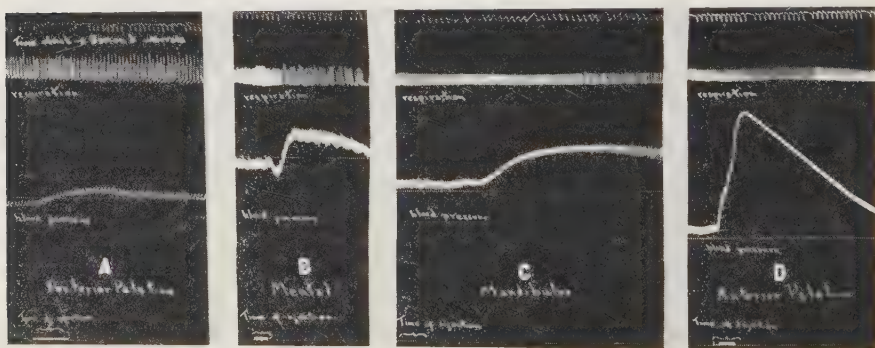


FIG. 1.

Effects in dogs of subgingival injections of procaine-epinephrine solutions on the blood pressure and the respiration when, A, the solution is injected into the posterior palatine region; B, into the mental foramen; C, into the region at the mandibular foramen; D, into the anterior palatine foramen.

² Koppányi, T., and Luckhardt, Arno B., *Arch. Int. de Pharm. et de Ther.*, 1931, **40**, 345.

The effects of these injections, as shown in Fig. 1, are representative of the results obtained in this series of experiments and as can be seen from the records, appreciable elevation of the general carotid blood pressure followed these subgingival injections. The most striking result is observed following injections in the region of the anterior palatine foramen in the soft tissues over the hard palate (D). The great increase in blood pressure from injections in this area as compared with any other injection was uniform throughout this second series. To meet the objection that these rises might have been due to reflex effects because of the pressure necessary for injection of the solution, physiological saline in the same and in much greater quantities was deposited, showing no pressor response.

Descriptive anatomy, Ellenberger and Baum,³ of the afferent and efferent vessels of the areas under observation, indicates a slightly greater vascularity in the anterior palatine region as compared with the posterior palatine region. A report by Schwartz⁴ suggests, furthermore, that there is a difference in sensibility of blood vessels to the local constrictor effect of epinephrine, which might account for the rather uniform differences noted in Figures 1A and 1D.

In studying the effects of subgingival injection of procaine-epinephrine, we cannot refrain from pointing out, even in this preliminary report, the implication of the results so far obtained, namely: (1) That subgingival injections of even dilute concentrations of epinephrine (1/30,000) may and do often cause marked rises in the blood pressure. (2) That if epinephrine, a local vasoconstrictor, reaches the general circulation in appreciable quantities so does procaine which itself is a vaso-dilator. (3) That in dental practice the patient is given, commonly, the same or repeated doses of the same concentration of procaine-epinephrine solutions irrespective of age or size of the individual and without previous physical examination of the patient as to the presence of a myocardial insufficiency, hypertension, or arteriosclerosis.

³ Ellenberger, W., and Baum, H., *Anatomie des Hundes*, Verlag von Paul Parey, Berlin, 1891, pp. 376-387.

⁴ Schwartz, M. S., *Dental Items of Interest*, 1937, **59**, 851.

9714 P

Nicotinic Acid and Prevention of Nutritional Panmyelophthisis in Rats.

PAUL GYÖRGY.

From the Babies and Childrens Hospital, and the Department of Pediatrics, School of Medicine, Western Reserve University, Cleveland.

Recently attention has been focused from different angles on nicotinic acid and nicotinamide. The importance of nicotinic acid in tissue metabolism as a co-enzyme^{1, 2} and as an agent for promoting growth in bacteria^{3, 4} has been recognized. Furthermore, Funk⁵ stated that nicotinic acid, especially nicotinamide, produced in rats and pigeons kept on a diet devoid of the vitamin B₂ complex "a much larger food intake and better weight, as compared with the controls." Even in the absence of substantiating details, the latter observations made it probable that nicotinic acid might constitute a separate factor in the vitamin B₂ complex.

While in my own unpublished experiments nicotinic acid had no effect in acrodynia in rats (vitamin B₆ deficiency disease⁶) either on the skin manifestations or on the growth rate, Elvehjem and his coworkers⁷ have succeeded in demonstrating the specific antiblack-tongue activity of nicotinic acid and nicotinamide in dogs. The therapeutic effect of nicotinic acid is not confined to canine black-tongue; it has been shown^{6, 8} to apply equally well to human pellagra. Thus the long-assumed pathogenetic identity of these 2 diseases is finally proved. In the light of these observations nicotinic acid and its derivative, nicotinamide, can be accepted as the specific antiblack-tongue or pellagra-preventing factor. This conclusion bears out the circumstantial evidence presented in previous experiments,⁹ according to which vitamin B₆ and the P-P factor must represent different constituents of the vitamin B₂ complex.

¹ Euler, H. v., Albers, H., and Schlenk, F., *Z. physiol. Chem.*, 1935, **234**, I; 1936, **240**, 113.

² Warburg, O., Christian, W., and Griese, A., *Biochem. Z.*, 1935, **279**, 143.

³ Knight, B. C. J. G., *Biochem. J.*, 1937, **31**, 731.

⁴ Mueller, J. H., *J. Bact.*, 1937, **34**, 429.

⁵ Funk, C., and Funk, J. C., *J. Biol. Chem.*, 1937, **119**, xxxv.

⁶ Cf. also Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, T. H., *Proc. Soc. Exp. Biol. and Med.*, 1937, **37**, 405.

⁷ Elvehjem, C. A., Madden, R. J., Strong, F. M., and Woolley, D. W., *J. Am. Chem. Soc.*, 1937, **59**, 1767.

⁸ Smith, D. T., Ruffin, J. M., and Smith, S. G., *J. A. M. A.*, 1937, **109**, 2054.

⁹ Birch, T. W., György, P., and Harris, L. J., *Biochem. J.*, 1935, **29**, 2830.

Rats are not very sensitive to a deficiency of the P-P factor. The only symptom that became noticeable⁹ in rats fed for 4 months diets that produced blacktongue in dogs or pellagra in human beings was a subnormal growth rate. Skin lesions or other typically pellagra-like manifestations have never been encountered.

In vitamin studies it is more suitable to use as the experimental basis a disease arising from deficiency of only one vitamin factor rather than a disease due to a complex deficiency. Thus in research having to do with the isolation of vitamin B₆ it seemed desirable to supplement the B₆ free basal diet, containing only vitamin B₁ and riboflavin as components of the whole vitamin B group, with nicotinic acid, in order to eliminate a disturbing although apparently not imposing complication which might be due to a deficiency of nicotinic acid.

This change in the diet (1000 gm. of food¹⁰ mixed with 1 gm. of nicotinic acid*) took place on Nov. 4, 1937, and involved the 180† rats fed the vitamin B₆ deficient diet. All other experimental conditions, and in particular the ingredients of the diet, remained the same as heretofore. As was expected, no difference has been observed in the production or the progress of the rat acrodynia and in the rate of growth of the rats in the experiment. However, the addition of nicotinic acid to the diet surprisingly resulted in *complete suppression* of panmyelophthisis, a complication which was a common occurrence¹¹ in rats kept under the same nutritional conditions but without nicotinic acid. In the 8 weeks preceding the addition of nicotinic acid, 20 fatalities due to panmyelophthisis were observed, while in the 8 weeks following the change in diet not one animal died from this disease, and, with the exception of one case of anemia (remarkable for the absence of agranulocytosis), all symptoms characteristic of panmyelophthisis were conspicuously absent after the second week. The rat with anemia has shown complete recovery on the diet plus nicotinic acid without any further specific supplement.¹¹ Dis-

TABLE I.

	Deaths from panmyelophthisis during week							
	1	2	3	4	5	6	7	8
Before								
addition of nicotinic acid to the diet	3	1	3	5	2	2	2	2
After	0	0	0	0	0	0	0	0

¹⁰ György, P., *Biochem. J.*, 1935, **29**, 741.

* Kindly furnished by the S.M.A. Corporation, Cleveland.

† This number fluctuated slightly, owing to intercurrent deaths and addition of new animals during the experiment.

¹¹ György, P., Goldblatt, H., Miller, F. R., and Fulton, R. P., *J. Exp. Med.*, 1937, **66**, 579.

tribution of the 20 fatalities over the 8 weeks in question was fairly uniform (Table I).

In previous studies,¹¹ panmyelophthisis has been found to be a disease with seasonal predilections, but November and December represented months in which panmyelophthisis was a frequent complication. In the experiments described here, the total number of animals was essentially the same in the 2 periods before and after addition of nicotinic acid to the diet.

In view of these facts the observations here recorded seem to indicate in rats a close relationship between nicotinic acid and prevention of nutritional panmyelophthisis with all its manifestations.

Further experiments are required in order to establish definitely the prophylactic, and possibly also the therapeutic effect of nicotinic acid on the maturation of bone marrow.

9715 P

Preparation and Administration of Globulin from Rabbit Antipneumococcus Sera.

MICHAEL HEIDELBERGER, JOSEPH C. TURNER AND CHECK M. SOO
Hoo.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York.

Although rabbit antipneumococcus sera offer certain theoretical^{1, 2, 3} and practical⁴ advantages in pneumonia therapy their use has not been without danger, so that a simple, though incomplete, method of antibody purification might be of value. The following method of preparing globulin for therapeutic purposes has been in effective use at the Presbyterian Hospital and is novel only in the substitution of efficient centrifugation for dialysis in the removal of the sodium sulfate used for precipitating the globulin. Analyses, data on administration, and excerpts from patients' charts are given.

Sera and solutions were kept sterile; the water and 0.85% saline

¹ Goodner, K., Horsfall, F. L., Jr., and Bauer, J. H., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 617.

² Heidelberg, M., Pedersen, K. O., and Tiselius, A., *Nature*, 1936, **138**, 165; Heidelberg, M., and Pedersen, K. O., *J. Exp. Med.*, 1937, **65**, 393.

³ Heidelberg, M., and Kendall, F. E., *J. Exp. Med.*, 1937, **65**, 647.

⁴ Goodner, K., Horsfall, F. L., Jr., and Dubos, R. J., *J. Immunol.*, 1937, **33**, 279, and references given there.

solution used were prepared with the precautions essential for safe intravenous use. Anhydrous sodium sulfate of the highest purity was sterilized by heat before use. The rabbit sera were allowed to stand in the cold for some weeks and freed from separated lipids and sediment. Antisera of a single type, containing 1.0 mg. of type-specific anticarbohydrate nitrogen* or more were pooled, diluted when necessary to reduce the antibody N content to 1.0 to 1.5 mg. per ml., and precipitated at 35-38°C. with an equal volume of filtered sodium sulfate solution saturated at the same temperature. After centrifugation at 3000-3500 r.p.m. the clear supernatant was drawn off and discarded; the precipitate was broken up and centrifuged again. The precipitate was washed with warm 60% saturated sodium sulfate solution and was repeatedly broken up and centrifuged until no more liquid could be obtained. The precipitate was dissolved in water, run through an ignited Chamberland L2 filter, and rinsed through with 0.85% saline solution. 1% by volume of 1% Merthiolate† was finally added. The solution may be heated at 56°. Types I, II, and III antipneumococcus rabbit sera have been fractionated in this way.

TABLE I.
Data on Antibody Globulin Solutions.

Solution	Anti-pneumococcus Type	Antibody N in sera taken mg.	Antibody N recovered mg.	Total N	Type sp. precipitin N
				in antibody globulin solution mg. per ml.	mg. per ml.
1	I	211	161	5.39	1.31
2	I	640	532	6.74	1.48
3	II	193	171	4.18	1.07
4	III	343	290		1.36
5	III	190	153	5.41	1.02
6	I	214	166	2.42	0.94

We have been able to give full therapeutic doses of antibody within 2 hours without untoward reaction by diluting the requisite amount of globulin solution to 500 ml. with 0.9% saline and administering this as a continuous, slow intravenous infusion. Results in 5 cases are shown in Table II (on following page).

* Analyses according to (3) and earlier papers.

† Manufactured by Eli Lilly and Son, Indianapolis, Ind.

TABLE II.
Excerpts from Patients' Charts.

Patient	Pn. Type	Bacteremia	Administration of antibody (in 500 ml. saline)	Reaction	Result
F.H.	III	+	12/22/37 75 ml. (102 mg. antibody N) 40 min.	Mild chill. T 102°*	Blood culture sterile 12/23 Recovered
G.B.	I	+	12/23/37 105 ml. (143 mg. antibody N) 95 min.	None	
			12/24/37 50 ml. (74 mg. antibody N) 100 min.	"	
			12/24/37 50 ml. (74 mg. antibody N) 120 min.	"	
			12/25/37 50 ml. (74 mg. antibody N) 135 min.	"	
			1/2/38 50 ml. (74 mg. antibody N) 220 min.	Slight urticaria. No rise in T.	
E.M.	I	0	1/2/38 50 ml. (74 mg. antibody N) 210 min.	None	Recovered
R.B.	I	+	1/18/37 50 ml. (65 mg. antibody N) undiluted, in divided doses	" †	Empyema
			1/19/37 50 ml. (65 mg. antibody N) as on 1/18	"	
			1/20/37 60 ml. (79 mg. antibody N) as on 1/18	"	
			1/21/38 100 ml. (102 mg. antibody N) 1000 ml. saline, 90 min.	Sl. respiratory dis- comfort. No rise in T.	
A.R.	III	0	1/22/38 37 ml. (38 mg. antibody N) 150 ml. saline, 30 min.	None	Blood culture sterile 1/21/37; recovered Under observation 1/23/38

*Infusion was given more quickly than in the next two cases.

†The antibody solution (Table I, No. 1) was not heated to 56° before use; horse serum was later given as skin test, with polysaccharide was negative and no more rabbit solution was available.

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